

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

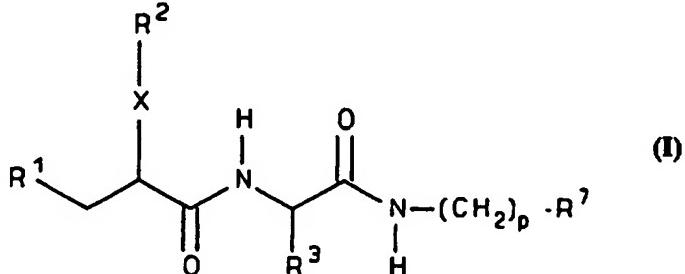
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07C 259/06, 237/22, C07F 9/60, C07C 327/30, 323/60, C07D 263/26, A61K 31/16, 31/66		A1	(11) International Publication Number: WO 96/16027 (43) International Publication Date: 30 May 1996 (30.05.96)
(21) International Application Number: PCT/US95/15530		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).	
(22) International Filing Date: 21 November 1995 (21.11.95)		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(30) Priority Data: 08/343,158 22 November 1994 (22.11.94) US			
(71) Applicants: SYNTEX (U.S.A.) INC. [US/US]; 3401 Hillview Avenue, Palo Alto, CA 94304 (US). AGOURON PHARMACEUTICALS, INC. [US/US]; 10350 North Torrey Pines Road, La Jolla, CA 92057 (US).			
(72) Inventors: CASTELHANO, Arlindo, L.; 3 Eagle Court, New City, NY 10956 (US). BENDER, Steven, L.; 606 Eucalyptus Street, Oceanside, CA 92054 (US). DEAL, Judith, G.; 45370 Zuma Drive, Temecula, CA 92592 (US). HORNE, Stephen; 2079 Victoria Avenue, Burlingame, Ontario L7R 1R5 (CA). LIAK, Teng, J.; 1609 Squall Court, Mississauga, Ontario L5M 4R6 (CA). YUAN, Zhengyu; 3410 Central Expressway, Santa Clara, CA 95051 (US).			
(74) Agents: KURZ, Walter et al.; Heller Ehrman White & McAuliffe, 525 University Avenue, Palo Alto, CA 94301-1900 (US).			

(54) Title: MATRIX METALLOPROTEASE INHIBITORS

(57) Abstract

Compounds of formula (I) and their pharmaceutically acceptable salts inhibit matrix metalloproteases, such as stromelysin, gelatinase, matriisin and collagenase, and are useful in the treatment of mammals having disease-states alleviated by the inhibition of such matrix metalloproteases.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

5

MATRIX METALLOPROTEASE INHIBITORS**FIELD OF THE INVENTION**

10 The present invention is directed to compounds and their pharmaceutically acceptable salts, which inhibit matrix metalloproteases, and are therefore useful in the treatment of mammals having disease-states alleviated by the inhibition of such matrix metalloproteases.

15

BACKGROUND OF THE INVENTION

Matrix metalloproteases ("MMPs") are a family of proteases (enzymes) involved in the degradation and remodeling of connective tissues. Members of this family of endopeptidase enzymes are present in various cell types that reside in or are associated with connective tissue, such as fibroblasts, monocytes, macrophages, endothelial cells, and invasive or metastatic tumor cells. MMP expression is stimulated by growth factors and cytokines in the local tissue environment, where these enzymes act to specifically degrade protein components of the extracellular matrix, such as collagen, proteoglycans (protein core), fibronectin and laminin. These ubiquitous extracellular matrix components are present in the linings of joints, interstitial connective tissues, basement membranes, and cartilage. Excessive degradation of extracellular matrix by MMPs is implicated in the pathogenesis of many diseases, including rheumatoid arthritis, osteoarthritis, periodontal disease, aberrant angiogenesis, tumor invasion and metastasis, corneal ulceration, and in complications of diabetes. MMP inhibition is, therefore, recognized as a good target for therapeutic intervention.

30 The MMPs share a number of properties, including zinc and calcium dependence, secretion as zymogens, and 40-50% amino acid sequence homology. The MMP family includes collagenases, stromelysins, gelatinases, and matrilysin, as discussed in greater detail below.

-2-

Interstitial collagenases catalyze the initial and rate-limiting cleavage of native collagen types I, II, III and X. Collagen, the major structural protein of mammals, is an essential component of the matrix of many tissues, for example, cartilage, bone, tendon and skin. Interstitial collagenases are very specific matrix metalloproteases which cleave collagen to give two fragments which spontaneously denature at physiological temperatures and therefore become susceptible to cleavage by less specific enzymes. Cleavage by the collagenase results in the loss of structural integrity of the target tissue, essentially an irreversible process.

The gelatinases include two distinct, but highly related, enzymes: a 72-kD enzyme secreted by fibroblasts and a wide variety of other cell types, and a 92-kD enzyme released by mononuclear phagocytes, neutrophils, corneal epithelial cells, tumor cells, cytотrophoblasts and keratinocytes. These gelatinases have been shown to degrade gelatins (denatured collagens), collagen types IV (basement membrane) and V, fibronectin and insoluble elastin.

The stromelysins (1 and 2) have been shown to cleave a broad range of matrix substrates, including laminin, fibronectin, proteoglycans, and collagen types IV and IX in their non-helical domains.

Matrilysin (putative metalloprotease or PUMP) is a recently described member of the matrix metalloprotease family. Matrilysin has been shown to degrade a wide range of matrix substrates including proteoglycans, gelatins, fibronectin, elastin, and laminin. Its expression has been documented in mononuclear phagocytes, rat uterine explants and sporadically in tumors.

Inhibitors of MMPs provide useful treatments for diseases associated with the excessive degradation of extracellular matrix, such as arthritic diseases (rheumatoid arthritis and osteoarthritis), bone resorptive diseases (such as osteoporosis), the enhanced collagen destruction associated with diabetes, periodontal disease, corneal ulceration, ulceration of the skin, tumor invasion and metastasis, and

-3-

aberrant angiogenesis.

The design and uses of MMP inhibitors is described, for example, in *J. Enzyme Inhibition* (1987), Vol. 2, pp. 1-22; *Drug News & Prospectives* (1990), Vol. 3, No. 8, pp. 453-458; *Arthritis and Rheumatism* (1993), Vol. 36, No. 2, pp. 181-189; *Arthritis and Rheumatism* (1991), Vol. 34, No. 9, pp. 1073-1075; *Seminars in Arthritis and Rheumatism* (1990), Vol. 19, No. 4, Supplement 1 (February), pp. 16-20; *Drugs of the Future* (1990), Vol. 15, No. 5, pp. 495-508; and *J. Enzyme Inhibition* (1987), Vol. 2, pp. 1-22. MMP inhibitors are also the subject of various patents and patent applications, for example, U.S. Patent Nos. 5,189,178 (Galardy) and 5,183,900 (Galardy), European Published Patent Applications 0 438 223 (Beecham) and 0 276 436 (F. Hoffmann-La Roche), and Patent Cooperation Treaty International Applications 92/21360 (Merck), 92/06966 (Beecham) and 92/09563 (Glycomed).

- 4 -

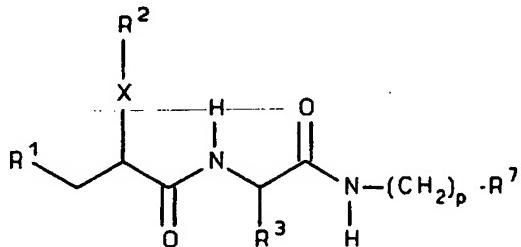
SUMMARY OF THE INVENTION

The invention provides new compounds which are useful as
 5 inhibitors of matrix metalloproteases and which are effective
 in treating disease-states characterized by excessive activity
 of matrix metalloproteases.

Accordingly, one aspect of the invention is directed to
 compounds of formula (I):

10

15



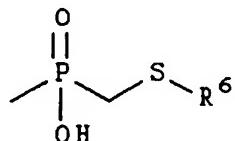
formula (I)

wherein:

20

R^1 is mercapto, acetylthio, carboxy, hydroxycarbamoyl,
 N-hydroxyformylamino, alkoxy carbonyl,
 aryloxycarbonyl, aralkoxycarbonyl,
 benzyloxycarbamoyl or a group of the formula

25



30

where R^6 is aryl or heteroaryl;

R^2 is alkyl, cycloalkyl, aryl, heterocycloalkyl, or
 heteroaryl;

R^3 is alkyl, cycloalkyl, aralkyl, or heteroaralkyl;

R^7 is aryl, heteroaryl or heterocycloalkyl;

X is a group of the formula $-(CH_2)_m-Y-(CH_2)_n-$, where:

35

Y is O, S, or a single bond,

m is an integer from 0 to 4,

n is an integer from 0 to 4, and

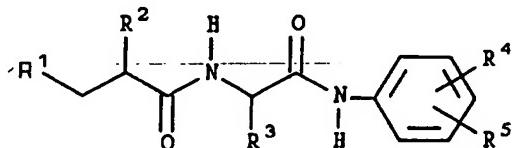
-5-

m + n is an integer from 0 to 4;
 p is an integer from 0 to 4, provided that R²-X is biphenylalkyl when p is not 0;
 and the pharmaceutically acceptable salts thereof.

5 Another aspect of the invention provides processes for synthesizing the compounds and salts of formula (I).

In another aspect, the invention is directed to a sub-genus of formula (I), i.e., the compounds of formula (II), as follows:

10



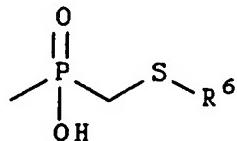
15

formula (II)

wherein:

R¹ is mercapto, acetylthio, carboxy, hydroxycarbamoyl, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, benzyl oxyaminocarbonyl or a group of the formula

20



25

where R⁶ is aryl or heteroaryl;

R² is alkyl, aralkyl or cycloalkylalkyl;

R³ is cycloalkyl, alkyl (optionally substituted by cycloalkyl, hydroxy, mercapto, alkylthio, aralkoxy, carboxy, amino, alkylamino, guanidino, carbamoyl, pyridyl or indolyl), or aralkyl (optionally substituted by hydroxy, carboxy, alkyl or alkoxy);

30

R⁴ is nitro, amino, cyano, hydroxy, alkoxy, carboxy, alkoxycarbonyl, alkylsulfonyl, haloalkyl, alkoxycarbonylalkyl, tetrazolyl, carbamoyl (optionally substituted by alkyl or

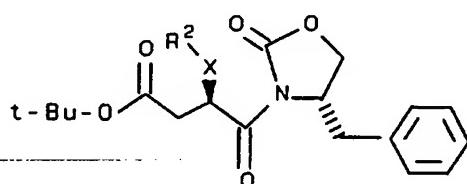
35

-6-

dialkylaminoalkyl), or aminosulfonyl (optionally substituted by alkyl); and
 R³ is hydrogen, halo or hydroxy,
 as a single stereoisomer or as a mixture thereof; and the
 pharmaceutically acceptable salts thereof.

5 Another aspect of the invention is directed to compounds of the formula

10



15

wherein:

R² is alkyl, aryl or heteroaryl; and

X is a group of the formula - (CH₂)_m-Y-(CH₂)_n, where:

Y is O, S, or a single bond,

m is an integer from 0 to 4,

20

n is an integer from 0 to 4, and

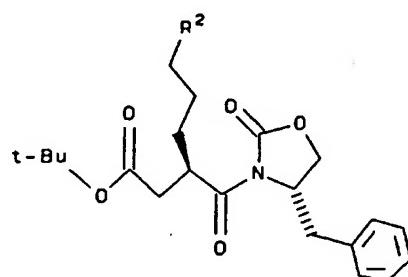
m + n is an integer from 0 to 4;

or R² and X together are lower alkenyl.

25

Another aspect of the invention is directed to processes for synthesizing a compound of the formula

30

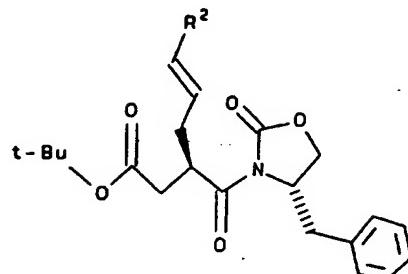


35

wherein R² is aryl or heteroaryl, by

- 7 -

(a) hydrogenating a compound of the formula:

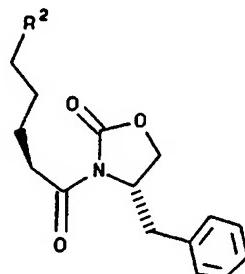


10

in the presence of a palladium/carbon catalyst; or

(b) contacting a compound of the formula

15



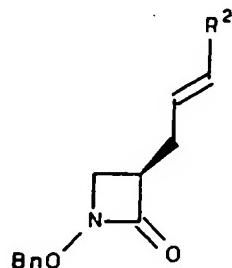
20

with sodium hexamethyldisilazide and *t*-butylbromoacetate.

25

Other aspects of the invention are directed to compounds of the formula

30



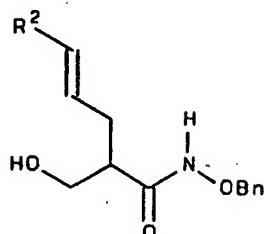
35

wherein *R*² is aryl or heteroaryl, and a process for synthesizing these compounds by

-8-

(a) contacting a compound of the formula:

5



10 wherein R² is hydrogen, aryl or heteroaryl, with an excess of mesyl chloride in pyridine followed by refluxing under basic conditions, and

15 (b) where R² is hydrogen in step (a), reacting the product of step (a) with an aryl halide or a heteroaryl halide in the presence of a base and a palladium catalyst.

20 Another aspect of the invention is directed to methods of inhibiting matrix metalloprotease activity in a mammal, which methods comprise administering to the mammal in need thereof a therapeutically effective amount of a compound of formula (I) as defined above, as a single stereoisomer, or as a mixture thereof, or a pharmaceutically acceptable salt thereof.

25 Another aspect of the invention is directed to a pharmaceutical composition useful in inhibiting matrix metalloprotease activity in a mammal, which composition comprises a therapeutically effective amount of a compound of formula (I) as defined above, as a single stereoisomer or as a mixture thereof; or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable excipient.

30

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used in the specification and appended claims, unless specified to the contrary, the following terms have the meaning indicated:

"BOC" refers to t-butoxycarbonyl.

"CBZ" refers to benzyloxycarbonyl (carbobenzyloxy).

-9-

"DCC" refers to *N,N*-dicyclohexylcarbodiimide.

"DMAP" refers to *N,N*-dimethylaminopyridine.

"DMF" refers to *N,N*-dimethylformamide.

5 "EDCI" refers to *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide.

"HOBT" refers to 1-hydroxybenzotriazole.

"Hydroxy" refers to the radical -OH.

"Amino" refers to the radical -NH₂.

10 "Acetylthio" refers to the radical -SC(O)CH₃.

"Halo" refers to bromo, chloro or fluoro.

"Carbamoyl" refers to the radical -C(O)NH₂.

"Carboxy" refers to the radical -C(O)OH.

"Hydroxyamino" refers to the radical -NHOH.

"Hydroxycarbamoyl" refers to the radical -C(O)NHOH.

15 "N-Hydroxyformylamino" refers to the radical -N(OH)C(O)H.

"Benzyloxycarbamoyl" refers to -C(O)N(H)OCH₂C₆H₅.

"Acylamino" refers to -NHC(O)R_a where R_a is alkyl.

"Mercapto" refers to the radical -SH.

20 "Alkyl" refers to a straight or branched chain monovalent radical consisting solely of carbon and hydrogen, containing no unsaturation and having from one to ten carbon atoms, e.g., methyl, ethyl, *n*-propyl, 2-methylpropyl (*iso*-butyl), 1-methylethyl (*iso*-propyl), *n*-butyl, and 1,1-dimethylethyl (*t*-butyl), heptyl and the like, which can be optionally substituted by cycloalkyl, hydroxy, mercapto, alkylthio, aralkoxy, carboxy, amino, mono- and di-alkylamino, guanidino, *N,N*-dialkylguanidino, carbamoyl, aryl, and heteroaryl.

25 "Alkane-diyl" or "alkylene" refers to a straight chain divalent radical consisting solely of carbon and hydrogen, containing no unsaturation and having from one to five carbon atoms, e.g., methylene, ethylene, propylene (or propane-1,3-diyl) and the like.

30 "Lower alkenyl" refers to a straight chain univalent hydrocarbon radical having from two to six carbon atoms and containing at least one unsaturated bond, e.g., prop-2-enyl, pent-4-enyl and the like.

35 "Alkylamino" refers to a radical of the formula -NHR_a

-10-

where R_a is alkyl as defined above, e.g., methylamino, ethylamino, iso-propylamino, n-butylamino, and the like.

"Haloalkyl" refers to a radical of the formula $-R_aR_d$ where R_a is alkyl as defined above substituted by one or more halo groups (R_d) as defined above, e.g., 2-chloroethyl, 2-bromoethyl, trifluoromethyl, and the like.

"Dialkylaminoalkyl" refers to a radical of the formula $-R_aN(R_a)_2$, where each R_a is independently an alkyl radical as defined above, e.g., dimethylaminoethyl, diethylamino-n-propyl, dimethylamino-n-propyl, and the like.

"Aminosulfonyl" refers to $-S(O)_2NH_2$.

"Alkylsulfonyl" refers to a radical of the formula $-S(O)_2R_a$ where R_a is alkyl as defined above, e.g., methylsulfonyl, ethylsulfonyl, iso-propylsulfonyl, and the like.

"Alkylsulfinyl" refers to a radical of the formula $-S(O)R_a$ where R_a is alkyl as defined above.

"Alkylthio" refers to a radical of the formula $-SR_a$ where R_a is optionally-substituted alkyl as defined above, e.g., methylthio, ethylthio, iso-propylthio, n-butylthio, and the like.

"Alkoxy" refers to a radical of the formula $-OR_a$ wherein R_a is alkyl as defined above, e.g., methoxy, ethoxy, n-propoxy, 1-methylethoxy, n-butoxy, t-butoxy, and the like.

"Alkoxycarbonylalkyl" refers to a radical of the formula $-R_aC(O)R_b$ where R_a is alkyl as defined above and R_b is alkoxy as defined above, e.g., methoxycarbonylethyl, ethoxycarbonylethyl, methoxycarbonyl-iso-propyl, and the like.

"Aryl" refers to a monovalent unsaturated aromatic carbocyclic radical having a single ring (e.g., phenyl), two condensed rings (e.g., naphthyl) or three condensed rings (e.g., phenanthrenyl or fluorenyl) which can be optionally substituted by one or more substituents independently selected from: alkyl, hydroxy, carboxy, halo, cyano, amino, nitro, tetrazolyl, heteroaryl, aminoalkoxy, alkylthio, haloalkyl, alkoxy, alkoxycarbonyl, alkoxycarbonylalkyl, alkyl sulfonyl,

-11-

alkyl sulfinyl, aminosulfonyl optionally substituted by alkyl, carbamoyl optionally substituted by alkyl or dialkylaminoalkyl, or the substituent can be another aryl group as defined herein (e.g., to form an optionally substituted biphenyl radical).

5 "Aryloxy" refers to a radical of the formula $-OR_b$ wherein R_b is aryl as defined above, e.g., phenoxy, quinol-2-yloxy, naphth-1-yloxy, or naphth-2-yloxy.

10 "Aralkyl" refers to a radical of the formula $-R_aR_b$ wherein R_a is alkyl as defined above and R_b is aryl as defined above, e.g., benzyl, phenylethylene, 3-phenylpropyl, and the like.

15 "Aralkoxy" refers to a radical of the formula $-OR_aR_b$ wherein R_a is alkyl as defined above and R_b is aryl as defined above, e.g., benzyloxy, 3-naphth-2-ylpropoxy, and the like.

20 "Alkoxycarbonyl" refers to a radical of the formula $-C(O)R_b$ wherein R_b is alkoxy as defined above, e.g., methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, and the like.

25 "Aralkoxycarbonyl" refers to a radical of the formula $-C(O)R_c$ wherein R_c is aralkoxy as defined above, e.g., benzyloxycarbonyl, and the like.

30 "Cycloalkyl" refers to a monovalent ring radical consisting solely of carbon and hydrogen atoms, containing no unsaturation and having from five to seven carbon atoms, e.g., cyclopentyl, cyclohexyl and cycloheptyl.

35 "Cycloalkylalkyl" refers to a radical of the formula $-R_eR_a$ where R_a is alkyl as defined above and R_e is cycloalkyl as defined above, e.g., cyclohexylmethyl, cyclohexylethyl, cyclopentylmethyl, and the like.

"Heteroaryl" refers to a monovalent unsaturated aromatic carbocyclic radical having a single ring or multiple condensed rings with at least one heteroatom such as N, O, S, (e.g., pyridyl, quinolyl, indolyl, carbazolyl, dibenzofuranyl, dibenzothiophenyl, phenanthridinyl), which can be optionally substituted by one or more substituents independently selected from: alkyl, hydroxy, carboxy, halo, cyano, amino, nitro,

-12-

5 tetrazolyl, aryl, aminoalkoxy, alkylthio, haloalkyl, alkoxy, alkoxycarbonyl, alkoxycarbonylalkyl, alkyl sulfonyl, alkyl sulfinyl, aminosulfonyl optionally substituted by alkyl, and carbamoyl optionally substituted by alkyl or dialkylaminoalkyl.

"Heteroaralkyl" refers to a radical of the formula -R_aR_b where R_a is alkyl as defined above and R_b is heteroaryl as defined above.

10 " Heterocycloalkyl" refers to a monovalent saturated carbocyclic radical having a single ring or multiple condensed rings with at least one heteroatom such as N,O,S (e.g., morpholino, piperazinyl, piperidinyl, pyrrolidinyl).

15 "Optional" or "optionally" means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted quinol-2-yl" means that the quinol-2-yl radical may or may not be substituted and that the description includes both substituted quinol-2-yl radicals and 20 quinol-2-yl radicals having no substitution.

25 "Amino-protecting group" as used herein refers to those organic groups intended to protect nitrogen atoms against undesirable reactions during synthetic procedures, and includes, but is not limited to, benzyl, acyl, acetyl, benzyloxycarbonyl (carbobenzyloxy), p-methoxybenzyloxy-carbonyl, p-nitrobenzyloxycarbonyl, t-butoxycarbonyl, trifluoroacetyl, and the like.

30 "Base" as used here includes both strong bases such as sodium hydroxide, lithium hydroxide, ammonium hydroxide, potassium carbonate and the like, and organic bases such as pyridine, diisopropylethylamine, N-methylmorpholine, triethylamine, dimethylaminopyridine and the like.

35 "Pharmaceutically acceptable salt" refers to those salts which retain the biological effectiveness and properties of the free bases or free acids and which are not biologically or otherwise undesirable. If the compound exists as a free base, the desired salt may be prepared by methods known to those of

-13-

ordinary skill in the art, such as treatment of the compound with an inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like; or with an organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. If the compound exists as a free acid, the desired salt may also be prepared by methods known to those of ordinary skill in the art, such as the treatment of the compound with an inorganic base or an organic base. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethyl-amino ethanol, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like.

"Mammal" includes humans and all domestic and wild animals, including, without limitation, cattle, horses, swine, sheep, goats, dogs, cats, and the like.

"Therapeutically effective amount" refers to that amount of a compound of formula (I) which, when administered to a mammal in need thereof, is sufficient to effect treatment, as defined below, for disease-states alleviated by the inhibition of matrix metalloprotease activity, such as the activity of stromelysin, gelatinase, matrilysin and/or collagenase. The amount of a compound of formula (I) which constitutes a

-14-

"therapeutically effective amount" will vary depending on the compound, the disease-state and its severity, and the mammal to be treated, but can be determined routinely by one of ordinary skill in the art having regard to his own knowledge
5 and to this disclosure.

"Treating" or "treatment" as used herein cover the treatment of a disease-state in a mammal, particularly in a human, which disease-state is alleviated by the inhibition of matrix metalloprotease activity, such as the activity of
10 stromelysin, gelatinase, matriLySIN and/or collagenase, and include:

- (i) preventing the disease-state from occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it;
- 15 (ii) inhibiting the disease-state, i.e., arresting its development; or
- (iii) relieving the disease-state, i.e., causing regression of the disease-state.

"Stereoisomers" refers to compounds having identical
20 molecular formulae and nature or sequence of bonding but differing in the arrangement of their atoms in space.

The compounds of formula (I), or their pharmaceutically acceptable salts, have at least two asymmetric carbon atoms in their structure, and may therefore exist as single
25 stereoisomers, racemates, and as mixtures of enantiomers and diastereomers. All such single stereoisomers, racemates and mixtures thereof are intended to be within the scope of this invention.

When naming the single stereoisomers of compounds of
30 formula (I) an absolute descriptor, R or S, may be assigned to the chiral carbon atoms therein according to the "Sequence Rule" procedure of Cahn, Ingold and Prelog.

Nomenclature

35 The nomenclature used herein is a modified form of I.U.P.A.C. nomenclature wherein the compounds of the invention are named as peptide derivatives. Where R³ of Formula (I)

-15-

comprises the side chain of an amino acid residue, that portion of the chemical structure which includes R³ together with the adjacent nitrogen atom (illustrated below and named as the N nitrogen, as opposed to the N' nitrogen) and carbonyl group is given the name of the corresponding amino acid. The naming and numbering of the compounds of the present invention is illustrated below for representative compounds of formula (I).

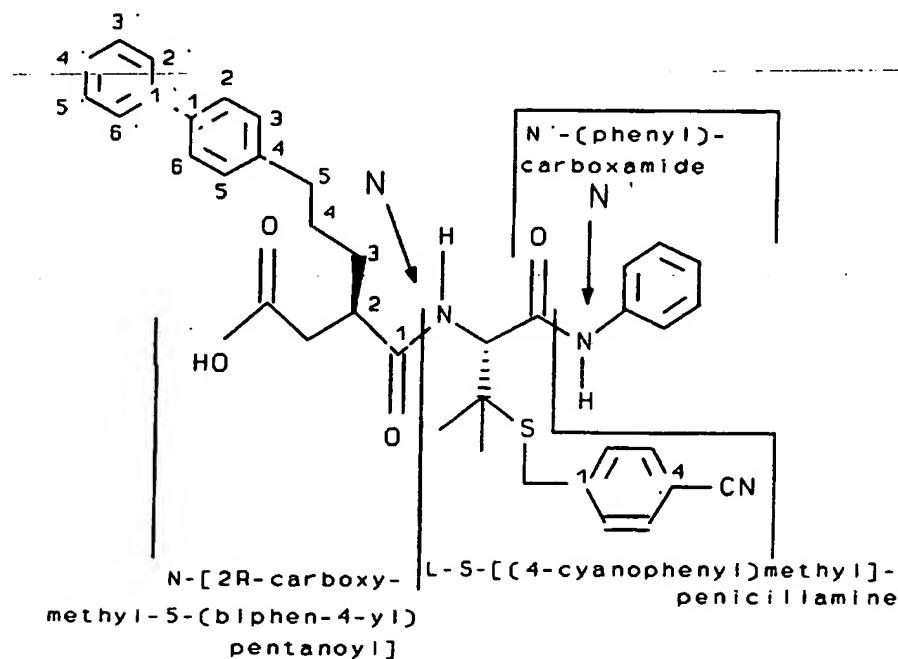
For example, the following compound of formula (I)

10

15

20

25



wherein R¹ is carboxy; R² is biphenyl; R³ is 4-(cyano)benzylthioisopropyl; R⁷ is phenyl; X is propane-1,3-diyil; and p is 0, is named

N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-S-((4-cyanophenyl)methyl)-penicillamine-N'-(phenyl)carboxamide. Another name for this compound is N-(5-(biphen-4-yl)-2R-carboxymethylpentanoyl)-L-S-((4-cyanophenyl)methyl)-penicillamine-N'-(phenyl)carboxamide.

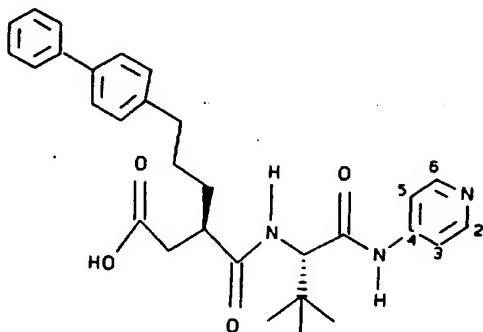
For ease of reference, the portions of the structure are associated with their corresponding nomenclature.

-16-

The structures and names of several other representative compounds of formula (I) follow.

5

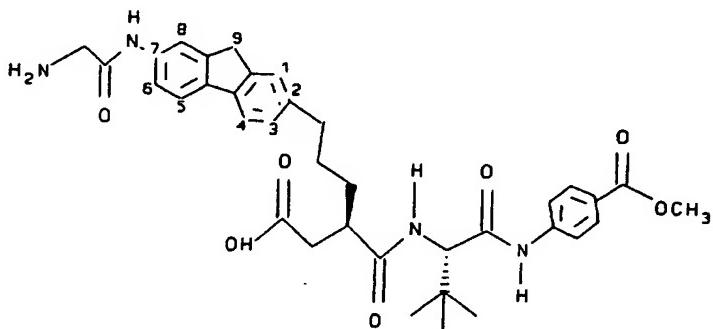
10



The above compound is named *N*-(2*R*-carboxymethyl-5-(biphen-4-yl)pentanoyl)-*L*-*t*-butylglycine-*N'*-(pyridin-4-yl)carboxamide. The term *t*-leucine can be interchanged with *t*-butylglycine, and the term pyridinyl can be interchanged with pyridyl. Another name for the above compound is: *N*-(5-biphen-4-yl-2*R*-carboxymethylpentanoyl)-*L*-*t*-leucine-*N'*-(pyridin-4-yl)-carboxamide.

25

30



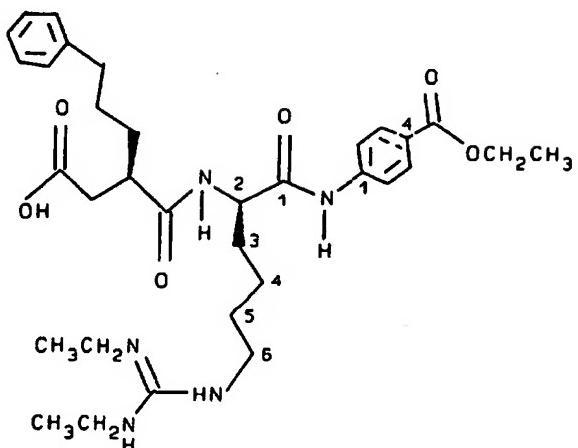
The above compound is named *N*-(2*R*-carboxymethyl-5-(7-(glycyl)aminofluoren-2-yl)pentanoyl)-*L*-leucine-*N'*-(4-(methoxycarbonyl)phenyl)carboxamide.

35

Another name for this compound is *N*-(5-(7-(glycyl)aminofluoren-2-yl)-2*R*-carboxymethylpentanoyl)-*L*-leucine-*N'*-(4-(methoxycarbonyl)phenyl)carboxamide.

-17-

5



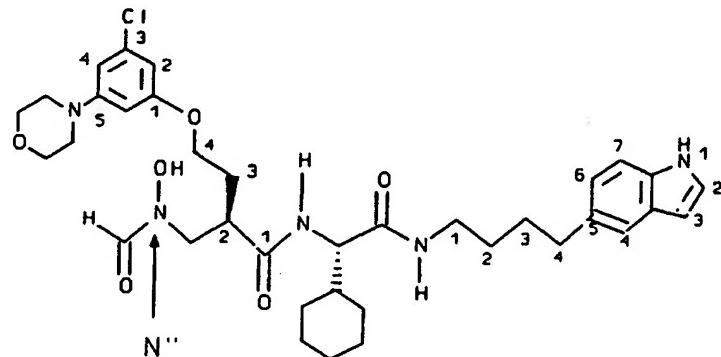
10

The above compound is named *N*-((2*R*-carboxymethyl-5-phenyl)pentanoyl)-L-6-(*N,N'*-diethylguanido)lysyl-*N'*-(4-(ethoxycarbonyl)phenyl)carboxamide. The term guanidino can be used interchangeably with guanido. Another name for this compound is *N*-((5-phenyl-2*R*-carboxymethyl)pentanoyl)-L-6-(*N,N'*-diethylguanidino)lysyl-*N'*-(4-(ethoxycarbonyl)phenyl)-carboxamide.

20

25

30



35

The above compound is named *N*-((2*R*-(*N''*-formyl-*N''*-hydroxyamino)methyl)-4-(((3-chloro-5-morpholino)phen-1-yl)oxybutanoyl)-L-cyclohexylglycine-*N'*(4-(indol-5-yl)-butyl)carboxamide. Another name for this compound is *N*-(4-(3-chloro-5-morpholino)phen-1-yl)-2*R*-((*N''*-formyl-*N''*-

-18-

hydroxyamino)methyloxybutanoyl)-L-cyclohexylglycine-N'-
(4-(indol-5-yl)butyl)carboxamide.

5

Utility, Testing and Administration

Utility

The compounds of formula (I) inhibit mammalian matrix metalloproteases, such as the stromelysins, gelatinases, matrilysin and collagenases, and are therefore useful for 10 treating diseases associated with the MMP-induced excessive degradation of matrix and connective tissue within the mammal, for example, arthritic diseases (rheumatoid arthritis and osteoarthritis), bone resorptive diseases (such as 15 osteoporosis), the enhanced collagen destruction associated with diabetes, periodontal disease, corneal ulceration, ulceration of the skin, tumor invasion and metastasis, and aberrant angiogenesis.

Testing

20

The ability of the compounds of formula (I) to inhibit matrix metalloprotease activity, such as the activity of stromelysin, gelatinase, matrilysin and/or collagenase may be demonstrated by a variety of *in vitro* and *in vivo* assays known to those of ordinary skill in the art, such as the assay 25 described in *Anal. Biochem.* (1985), Vol. 147, p. 437, and the MMP Enzymatic Assay described in *FEBS* (1992), Vol. 296(3), p. 263, or modifications thereof.

Administration

30

Administration of the compounds of formula (I), or their pharmaceutically acceptable salts, in pure form or in an appropriate pharmaceutical composition, can be carried out via any of the accepted modes of administration or agents for serving similar utilities. Thus, administration can be, for example, orally, nasally, parenterally, topically, transdermally, or rectally, in the form of solid, semi-solid, lyophilized powder, or liquid dosage forms, such as for 35

-19-

example, tablets, suppositories, pills, soft elastic and hard gelatin capsules, powders, solutions, suspensions, or aerosols, or the like, preferably in unit dosage forms suitable for simple administration of precise dosages. The compositions will include a conventional pharmaceutical carrier or excipient and a compound of formula (I) as the/an active agent, and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc.

Generally, depending on the intended mode of administration, the pharmaceutically acceptable compositions will contain about 1% to about 99% by weight of a compound(s) of formula (I), or a pharmaceutically acceptable salt thereof, and 99% to 1% by weight of a suitable pharmaceutical excipient. Preferably, the composition will be about 5% to 15% by weight of a compound(s) of formula (I), or a pharmaceutically acceptable salt thereof, with the rest being suitable pharmaceutical excipients.

The preferred route of administration is oral, using a convenient daily dosage regimen which can be adjusted according to the degree of severity of the disease-state to be treated. For such oral administration, a pharmaceutically acceptable composition containing a compound(s) of formula (I), or a pharmaceutically acceptable salt thereof, is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, starch, pregelatinized starch, magnesium stearate, sodium saccharine, talcum, cellulose ether derivatives, glucose, gelatin, sucrose, citrate, propyl gallate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like.

Preferably such compositions will take the form of capsule, caplet or tablet and therefore will also contain a diluent such as lactose, sucrose, dicalcium phosphate, and the like; a disintegrant such as croscarmellose sodium or derivatives thereof; a lubricant such as magnesium stearate and the like; and a binder such as a starch, gum acacia,

-20-

polyvinylpyrrolidone, gelatin, cellulose ether derivatives, and the like.

5 The compounds of formula (I), or their pharmaceutically acceptable salts, may also be formulated into a suppository using, for example, about 0.5% to about 50% active ingredient disposed in a carrier that slowly dissolves within the body, e.g., polyoxyethylene glycols and polyethylene glycols (PEG), e.g., PEG 1000 (96%) and PEG 4000 (4%).

10 Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc., a compound(s) of formula (I) (about 0.5% to about 20%), or a pharmaceutically acceptable salt thereof, and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol and the like, to thereby form a solution or suspension.

15 If desired, a pharmaceutical composition of the invention may also contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, antioxidants, and the like, such as, for example, citric acid, sorbitan monolaurate, triethanolamine oleate, butylated hydroxytoluene, etc.

20 Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's Pharmaceutical Sciences*, 18th Ed., 25 (Mack Publishing Company, Easton, Pennsylvania, 1990). The composition to be administered will, in any event, contain a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof, for treatment of a disease-state alleviated by the inhibition of matrix metalloprotease activity in accordance with the teachings of this invention.

30 The compounds of formula (I), or their pharmaceutically acceptable salts, are administered in a therapeutically effective amount which will vary depending upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of the compound, the age, body weight, general health, sex, diet,

-21-

mode and time of administration, rate of excretion, drug combination, the severity of the particular disease-state, and the host undergoing therapy. Generally, a therapeutically effective daily dose is from about 0.14 mg to about 14.3 mg/kg of body weight per day of a compound of formula (I), or a pharmaceutically acceptable salt thereof; preferably, from about 0.7 mg to about 10 mg/kg of body weight per day; and most preferably, from about 1.4 mg to about 7.2 mg/kg of body weight per day. For example, for administration to a 70 kg person, the dosage range would be from about 10 mg to about 1.0 gram per day of a compound of formula (I), or a pharmaceutically acceptable salt thereof, preferably from about 50 mg to about 700 mg per day, and most preferably from about 100 mg to about 500 mg per day.

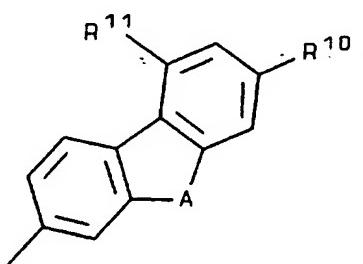
15

Preferred Embodiments

Preferred are the compounds of formula (I) where X is alkane-diyl and where p is zero, 2 or 3.

20 Of the compounds where p is 2 or 3, particularly preferred are those compounds where R¹ is carboxy, R² is biphenyl, R³ is cyclohexyl, and R⁷ is optionally substituted phenyl [especially 4-(aminosulfonyl)phenyl] or N-morpholino.

25 Of the compounds where p is zero, particularly preferred are the group of compounds where R² is alkyl, optionally substituted phenyl, or a group of the formula:



30

35

and R⁷ is 4-pyridyl or optionally substituted phenyl.

Within this group, a preferred subgroup of compounds are

-22-

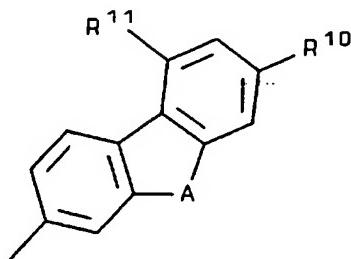
those where R¹ is carboxy, hydroxycarbamoyl, or N-hydroxy-formylamino; R² is phenyl, biphenyl, 4-(pyridyl)phenyl, or 2-methylpropyl; R³ is t-butyl, 4-aminobutyl, alkylaminobutyl, dialkylaminobutyl, 4-(N,N'-diethylguanidino)butyl, propyl, 2-methylpropyl, 1-hydroxyisopropyl, 1-hydroxyethyl, or cyclohexyl; and X is a single bond, ethylene or propane-1,3-diyl.

Within this subgroup, a preferred class of compounds are those where R² is biphenyl, R³ is t-butyl and R⁷ is 4-pyridyl, particularly where R¹ is carboxy, N-hydroxyformylamino, or hydroxycarbamoyl.

Also preferred is the subgroup of compounds where R² is a group of the formula:

15

20



wherein A is CH₂; R¹⁰ is H or acylamino; R¹¹ is H; R⁷ is optionally substituted phenyl; and X is propane-1,3-diyl.

Within this subgroup, preferred are the compounds where R¹ is carboxy, hydroxycarbamoyl, or N-hydroxyformylamino; R³ is alkyl (especially 2-methylpropyl); and R⁷ is alkoxy carbonylphenyl [especially 4-(methoxycarbonyl)phenyl].

30 Also within this group, preferred are the subgroup of compounds where R¹ is carboxy; R² is phenyl; R³ is alkyl [especially 4-(amino)butyl and 4-(diethylguanidino)-N-butyl] or cycloalkyl (especially cyclohexyl); and R⁷ is optionally substituted phenyl [especially 4-(ethoxycarbonyl)phenyl or 4-(dialkylaminoethylaminosulfonyl)phenyl]; and X is ethylene or propane-1,3-diyl.

Also within this group, preferred are the subgroup of

-23-

compounds where R¹ is mercapto, carboxy, hydroxycarbamoyl, or N-hydroxyformylamino; R² is 2-methylpropyl; R³ is alkyl [especially propyl, 2-methylpropyl], cycloalkyl [especially cyclohexyl] or heteroaralkyl [especially 3-methylindolyl]; R⁷ is optionally substituted phenyl [especially 4-methoxyphenyl, 4-carboxyphenyl, 4-(methoxycarbonyl)phenyl or 4-(dimethylaminoethylcarbamoyl)phenyl]; and X is a single bond.

Also within this group, preferred are the subgroup of compounds where R¹ is carboxy; R² is 4-(2-hydroxyethyl)phenyl, 4-(2-hydroxypropyl)phenyl, 4-(2-hydroxybutyl)phenyl, 4-(pyridyl)phenyl, biphenyl, 4'-(aminoethoxy)biphenyl, 4'-(cyano)biphenyl, or 4'-(hydroxy)biphenyl; R³ is 2-methylpropyl; R⁷ is 4-(methoxycarbonyl)phenyl; and X is propane-1,3-diyl.

Particularly preferred is the subgroup of compounds where R² is biphenyl, especially where R⁷ is optionally substituted phenyl.

Within this particularly preferred subgroup, preferred are the compounds where R¹ is carboxy; R³ is alkyl or cycloalkyl [especially cyclohexyl, 4-(amino)butyl, 4-(isopropylamino)butyl, 1-hydroxyisopropyl or t-butyl]; X is propane-1,3-diyl, and R⁷ is phenyl, 4-(hydroxyethylaminosulfonyl)phenyl, 4-(dimethylaminoethylamino sulfonyl)-phenyl, 4-(ethoxycarbonyl)phenyl, 4-(N-morpholinopropylamino-sulfonyl)phenyl, 4-(methylaminosulfonyl)phenyl, 4-(hydroxyethylaminosulfonyl)phenyl, or 4-(methylsulfinyl)phenyl.

Another preferred group, particularly for matrilysin inhibition, are the compounds of formula (II), particularly those compounds wherein R¹ is mercapto or acetylthio.

Within this second group, a preferred subgroup of compounds are those compounds wherein R² is alkyl, aralkyl, cycloalkylalkyl; R³ is cycloalkyl or alkyl (optionally substituted by cycloalkyl, hydroxy, aralkoxy, alkylthio, pyridyl or indolyl); R⁴ is cyano, carboxy, hydroxy, alkoxy, alkoxycarbonyl, alkoxycarbonylalkyl, carbamoyl (optionally substituted by aralkylaminoalkyl), or aminosulfonyl (optionally substituted by alkyl); and R⁵ is hydrogen.

-24-

Within this subgroup, a preferred class of compounds are those compounds wherein R² is alkyl; R³ is cyclohexyl, alkyl (optionally substituted by cyclohexyl, hydroxy, benzyloxy, methylthio, pyridyl or indolyl); and R⁴ is carboxy, alkoxycarbonyl and aminosulfonyl.

5 Within this class of compounds, compounds wherein R² is 2-methylpropyl are preferred. Particularly preferred are those compounds wherein R³ is 2-methylpropyl.

10 A third group preferred for matrilysin inhibition, are the compounds of formula (II) wherein R¹ is carboxy.

15 Within this third group, a preferred subgroup of compounds are those compounds wherein R² is alkyl, aralkyl, cycloalkylalkyl; R³ is cycloalkyl or alkyl (optionally substituted by cycloalkyl, hydroxy, aralkoxy, alkylthio, pyridyl or indolyl); R⁴ is cyano, hydroxy, alkoxy, carboxy, alkoxycarbonyl, alkoxycarbonylalkyl, carbamoyl (optionally substituted by aralkylaminoalkyl), or aminosulfonyl (optionally substituted by alkyl); and R⁵ is hydrogen.

20 Within this subgroup, a preferred class of compounds are those compounds wherein R² is alkyl; R³ is cyclohexyl, alkyl (optionally substituted by cyclohexyl, hydroxy, benzyloxy, methylthio, pyridyl or indolyl); and R⁴ is carboxy, alkoxycarbonyl and aminosulfonyl.

25 Within this class of compounds, preferred compounds are those compounds wherein R² is 2-methylpropyl. Particularly preferred are those compounds wherein R³ is cyclohexyl, 2-methylpropyl, pyridin-3-ylmethyl, 1-benzyloxyethyl, 1-methylpropyl, 1,1-dimethylethyl, 1-hydroxyethyl, and indol-2-ylmethyl; and R⁴ is methoxycarbonyl.

30 A fourth group preferred for matrilysin inhibition, are the compounds of formula (II) wherein R¹ is hydroxycarbamoyl.

35 Within this fourth group, a preferred subgroup of compounds are those compounds wherein R² is alkyl, aralkyl, cycloalkylalkyl; R³ is cycloalkyl or alkyl (optionally substituted by cycloalkyl, hydroxy, aralkoxy, alkylthio, pyridyl or indolyl); R⁴ is cyano, hydroxy, alkoxy, carboxy, alkoxycarbonyl, alkoxycarbonylalkyl, carbamoyl (optionally

-25-

substituted by aralkylaminoalkyl), or aminosulfonyl (optionally substituted by alkyl); and R⁵ is hydrogen.

Within this subgroup, a preferred class of compounds are those compounds wherein R² is alkyl; R³ is cyclohexyl, alkyl (optionally substituted by cyclohexyl, hydroxy, benzyloxy, methylthio, pyridyl or indolyl); and R⁴ is carboxy, alkoxycarbonyl and aminosulfonyl.

Within this class, preferred compounds are those compounds wherein R² is 2-methylpropyl. Particularly preferred are those compounds wherein R³ is cyclohexyl, 2-methylpropyl, pyridin-3-ylmethyl, 1-benzyloxyethyl, 1-methylpropyl, 1,1-dimethylethyl, 1-hydroxyethyl, and indol-2-ylmethyl.

Presently, the most preferred compounds of formula (I) are the following:

- 15 *N*-(2*R*-(*N*^{''}-hydroxycarbamoyl)methyl-4-(methyl)pentanoyl)-
 L-tryptophan-*N*'-(4-(carboxy)phenyl)carboxamide;
 N-(2*R*-(*N*^{''}-hydroxycarbamoyl)methyl-4-(methyl)pentanoyl)-
 L-leucine-*N*'-(4-(methoxycarbonyl)phenyl)carboxamide;
 N-(2*R*-(*N*^{''}-hydroxycarbamoyl)methyl-4-(methyl)pentanoyl)-
20 L-leucine-*N*'-(4-(carboxy)phenyl)carboxamide;
 N-(2*R*-mercaptomethyl-4-(methyl)pentanoyl)-
 L-leucine-*N*'-(4-(methoxycarbonyl)phenyl)carboxamide;
 N-(2*R*-acetylthiomethyl-4-(methyl)pentanoyl)-
 L-leucine-*N*'-(4-methoxycarbonylphenyl)carboxamide;
25 *N*-(2*R*-carboxymethyl-4-(methyl)pentanoyl)-
 L-leucine-*N*'-(4-(methoxycarbonyl)phenyl)carboxamide;
 N-(2*R*-(*N*^{''}-hydroxycarbamoyl)methyl-4-(methyl)pentanoyl)-
 L-cyclohexylglycine-*N*'-(4-(methoxycarbonyl)phenyl)-
 carboxamide;
30 *N*-(2*R*-(*N*^{''}-hydroxycarbamoyl)methyl-4-(methyl)pentanoyl)-
 L-t-leucine-*N*'-(4-(methoxycarbonyl)phenyl)carboxamide;
 N-(2*R*-(*N*-hydroxycarbamoyl)methyl-5-(biphen-4-yl)pentanoyl-
 L-t-leucine-*N*'-(pyrid-4-yl)carboxamide;
 N-(2*R*-carboxymethyl-5-(biphen-4-yl)pentanoyl)-
35 L-t-leucine-*N*'-(pyridin-4-yl)carboxamide;
 N-(2*R*-carboxymethyl-5-(biphen-4-yl)pentanoyl)-
 L-t-leucine-*N*'-(4-((2-hydroxyethyl)aminosulfonyl)-

-26-

- phenyl) carboxamide;
- N*- (2*R*-carboxymethyl-5- (4- (pyrid-4-yl) phenyl) pentanoyl) -
L-leucine-*N'*- (4- (methoxycarbonyl) phenyl) carboxamide;
- N*- (2*R*-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
5 L- β -hydroxyvaline-*N'*- (phenyl) carboxamide;
- N*- (*N*ⁿ-formyl-*N*ⁿ-hydroxyamino)methyl-5- (biphen-4-yl) pentanoyl) -
L-*t*-leucine-*N'*- (pyridin-4-yl) carboxamide;
- N*- (2*R,S*) - (*N*ⁿ-formyl-*N*ⁿ-hydroxyamino)methyl-4-
10 (methyl) pentanoyl) -
L-leucine-*N'*- (4- (methoxycarbonyl) phenyl) carboxamide;
- N*- (2*R*-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
L-*t*-leucine-*N'*- (4*R,S*- (methylsulfinyl) phenyl) carboxamide;
- N*- (2*R*-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
15 L-*t*-leucine-*N'*- (4- (methylaminosulfonyl) phenyl) -
carboxamide;
- N*- (2*R*-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
L-*t*-leucine-*N'*- (4- (3- (morpholin-4-yl) propylamino-
sulfonyl) phenyl) carboxamide;
- N*- (2*R*-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
20 L-cyclohexylglycine-*N'*- (4- ((2-hydroxyethyl) -
aminosulfonyl) phenyl) carboxamide;
- N*- (2*R*-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
L-cyclohexylglycine-*N'*- (4 ((2-(dimethylamino)ethyl) -
aminosulfonyl) phenyl) carboxamide;
- 25 *N*- (2*R*- (*N*ⁿ-hydroxycarbamoyl)methyl-4- (methyl) pentanoyl)
D,L-norvaline-*N'*- (4- (dimethylaminoethylcarbamoyl) phenyl) -
carboxamide;
- N*- (2*R*-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
L-lysine-*N'*- (4- (ethoxycarbonyl) phenyl) carboxamide;
- 30 *N*- (2*R*-carboxymethyl-5- (phenyl) pentanoyl) -
L- (N-lysine-*N'*- (4- (ethoxycarbonyl) phenyl) carboxamide;
- N*- (2*R*-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
L- (N ϵ -isopropyl) lysine-*N'*- (4- (ethoxycarbonyl) -
phenyl) carboxamide;
- 35 *N*- (2*R*-carboxymethyl) -4- (phenyl) butanoyl) -
L-cyclohexylglycine-*N'*- (4- (*N,N*-dimethyl-
aminoethylaminosulfonyl)-phenyl) carboxamide; and

-27-

N-(2*R*-carboxymethyl-5-(phenyl)pentanoyl)-
L-(*N,N'*-diethylguanido)lysine-*N*-(4-(ethoxy-
carbonyl)phenyl)carboxamide.

5

SYNTHESIS OF COMPOUNDS OF FORMULA (I)

The compounds of formula (I) are prepared as described below, for example with reference to Reaction Schemes 1-7, in which the substituent groups illustrated (e.g., R¹, R², etc.) have the same meanings as described in the Summary of the Invention, unless otherwise specified. Certain of the reaction schemes illustrate structures of formula (I) where p is zero and R⁷ is an optionally substituted phenyl group [the substituents R⁴ and R⁵ having been described in connection with formula (II) in the Summary of the Invention]. As those skilled in the art will appreciate, while the corresponding compounds where p is 1-4 and where R⁷ is as otherwise defined can be analogously prepared, combinations of substituents and/or variables in compounds of formula (I) and intermediates thereof are permissible only when such combinations result in stable compounds.

Compounds of formula (I) and their pharmaceutically acceptable salts, as single stereoisomers or as mixtures thereof, are peptide derivatives all or portions of which can be prepared from the constituent α-amino acid derivative(s). Standard methods for the formation of peptide bonds are illustrated by M. Bodanszky et al., *The Practice of Peptide Synthesis* (1984), Springer-Verlag; M. Bodanszky, *Principles of Peptide Synthesis* (1984), Springer-Verlag; J.P. Greenstein et al., *Chemistry of the Amino Acids* (1961), Vol. 1-3, John Wiley and Sons Inc.; G.R. Pettit, *Synthetic Peptides* (1970), Vol. 1-2, Van Nostrand Reinhold Company.

35 **Synthetic Reaction Parameters**

The terms "solvent", "inert organic solvent" or "inert solvent" mean a solvent inert under the conditions of the

-28-

reaction being described in conjunction therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform, methylene chloride (or dichloromethane), diethyl ether, methanol, 5 pyridine and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert organic solvents.

The term "q.s." means adding a quantity sufficient to achieve a stated function, such as to bring a solution to a 10 desired volume.

Unless specified to the contrary, the reactions described herein take place at atmospheric pressure within a temperature range from 5°C to 100°C (preferably from 10°C to 50°C; most preferably at "room" or "ambient" temperature, e.g., 20°C). 15 Further, unless otherwise specified, the reaction times and conditions are intended to be approximate, e.g., taking place at about atmospheric pressure within a temperature range of about 5°C to about 100°C (preferably from about 10°C to about 50°C; most preferably about 20°C) over a period of about 1 to 20 about 10 hours (preferably about 5 hours). Parameters given in the Examples are intended to be specific, not approximate.

Amide couplings used to form the compounds of formula (I) are generally performed by the carbodiimide method with reagents such as dicyclohexylcarbodiimide or *N'*-ethyl- 25 *N*-(3-dimethylaminopropyl)-carbodiimide (EDCI) in the presence of 1-hydroxybenzotriazole (HOBT) in an inert solvent such as dimethylformamide (DMF). Other methods of forming the amide or peptide bond include, but are not limited to synthetic routes via an acid chloride, acyl azide, mixed anhydride or activated ester such as nitrophenyl ester. Typically, 30 solution phase amide couplings with or without peptide fragments are performed.

The selection of protecting groups for the terminal amino or carboxy groups of compounds used in the preparation of the compounds of formula (I) is dictated in part by the particular amide or peptide coupling conditions, and in part by the amino acid and/or peptide components involved in the coupling. 35

-29-

Amino-protecting groups commonly used include those which are well known in the art, e.g., *p*-methoxybenzyloxycarbonyl, benzyloxycarbonyl (also referred to as carbobenzyloxy or CBZ), *p*-nitrobenzyloxycarbonyl, *t*-butoxycarbonyl (BOC), and the like. It is preferred to use either BOC or CBZ as the protecting group for the α -amino group because of the relative ease of its removal by mild acids [e.g., by trifluoroacetic acid (TFA) or hydrochloric acid in ethyl acetate] or by catalytic hydrogenation.

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples hereinbelow. However, other equivalent separation or isolation procedures can, of course, also be used.

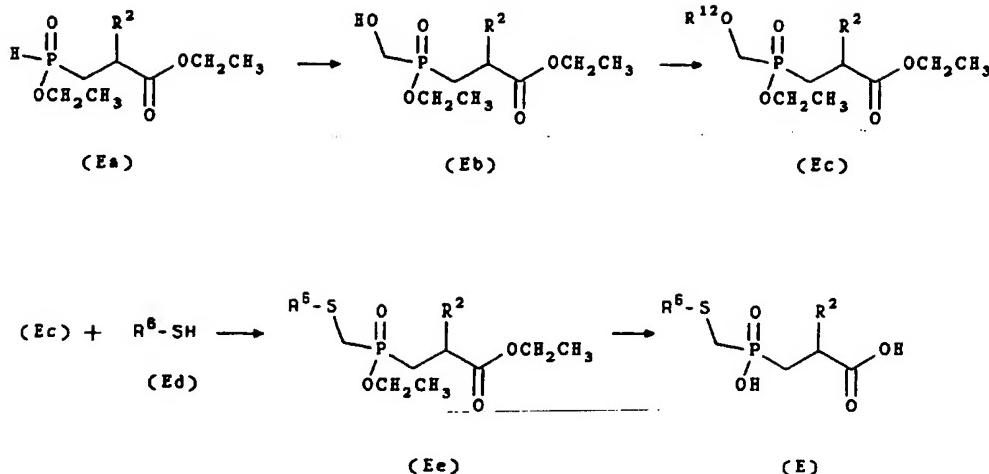
The individual stereoisomers of compounds of formula (I) may be separated from each other by methods known to those of ordinary skill in the art, e.g., by selective crystallization or by chromatography, and/or by the methods disclosed herein.

Preparation of Formula (E)

Compounds of formula (E) are intermediates used in the preparation of compounds of formula (I), and are prepared as shown in Reaction Scheme 1 wherein R¹² is mesyl or tosyl.

-30-

Reaction Scheme 1



5 Starting Materials

Compounds of formula (Ea) may be prepared according to methods known to those of ordinary skill in the art (e.g., see European Published Patent Application 0 276 436) or may be prepared according to the method described in Example 1 below. Compounds of formula (Ed) are commercially available or may be prepared according to methods known to those of ordinary skill in the art.

Formula (Eb) - In general, compounds of formula (E) are prepared by first treating a compound of formula (Ea) in an aprotic solvent, preferably tetrahydrofuran and methylene chloride, at 0-15°C, preferably at 0°C, in the presence of a base, preferably diisopropylethylamine and bis-(trimethylsilyl)acetamide, with paraformaldehyde. The resulting solution is brought to 25-37°C, preferably to 37°C, for 18 hours. The alcohol of formula (Eb) is then isolated by standard methods, preferably by evaporation of solvent, extraction and filtration.

Formula (Ec) - An alcohol of formula (Eb) in an aprotic solvent, preferably methylene chloride, is then cooled to -20°C to about 0°C, preferably to about -20°C, and is then

-31-

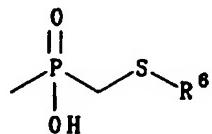
esterified by the standard procedure of treating the alcohol with at least a stoichiometric amount to about a 100% excess of either mesyl chloride or tosyl chloride. The esterification takes place over an initial period of time (preferably 15 minutes) at -20°C, followed by second period of time (preferably 3.5 hours) at room temperature. The ester of formula (Ec) is then isolated from the reaction mixture by standard isolation procedures, preferably by extraction, filtration and evaporation.

10 Formula (Ee) - An ester of formula (Ec) in an aprotic solvent, preferably DMF, is then reacted with a salt of a compound of formula (Ed) (preferably the sodium salt formed from the reaction of the compound of formula (Ed) with sodium hydride in an aprotic solvent, preferably DMF), for about 16-20 hours, preferably for about 18 hours, at temperatures beginning at about 0°C and slowly warming to room temperature. The resulting mercapto compound of formula (Ee) is isolated from the reaction mixture by standard isolation techniques, such as by extraction, evaporation, and flash chromatography.

15 Formula (E) - A compound of formula (Ee) is then hydrolyzed under basic conditions, preferably in the presence of sodium hydroxide, to form a compound of formula (E), which is isolated from the reaction mixture by standard isolation techniques.

20 **Preparation of Formula (Ia)**

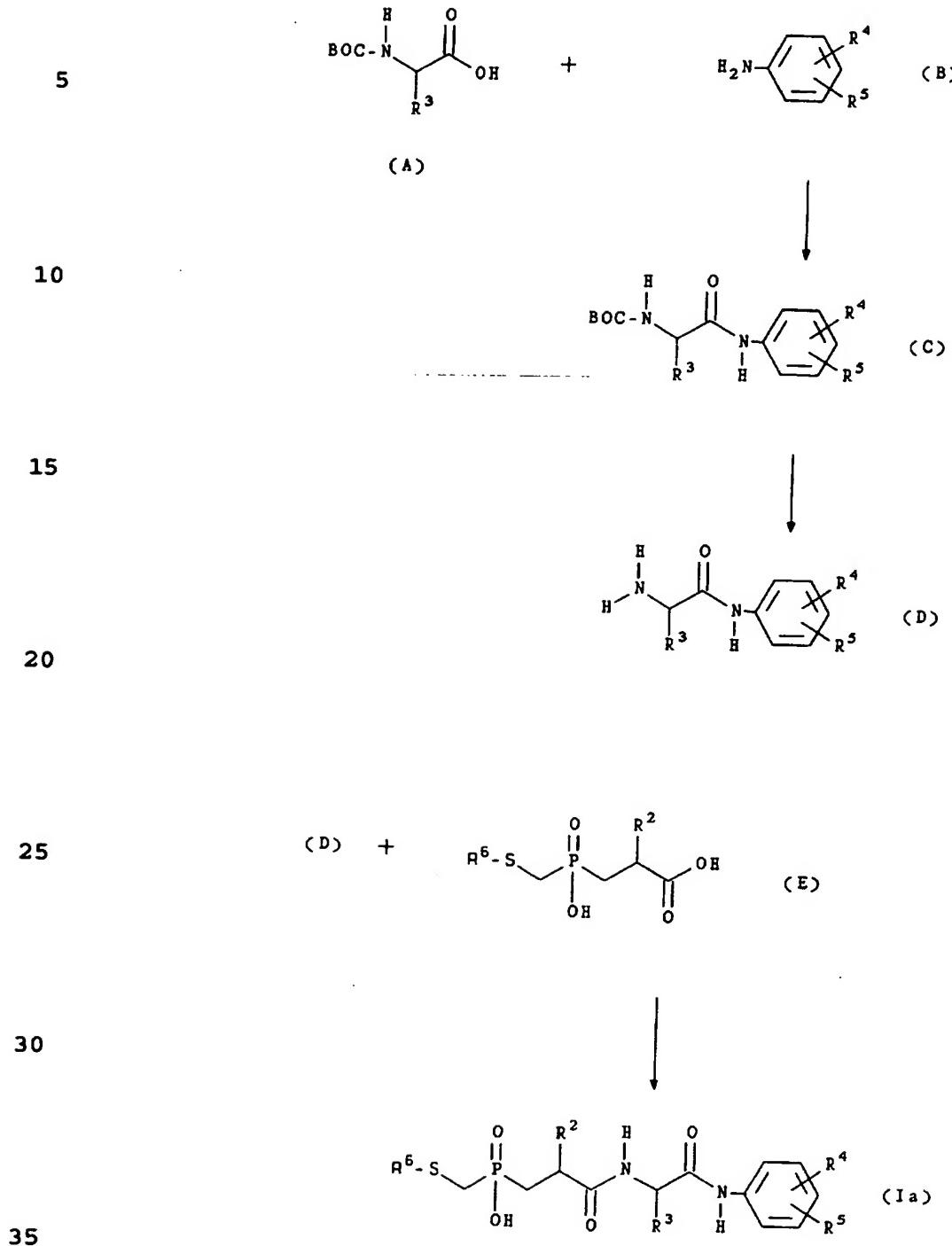
Compounds of formula (Ia) are compounds of formula (I) wherein R¹ is a group of the formula



35 (wherein, when R⁶ is aryl, it is preferably naphth-1-yl, naphth-2-yl or phenyl, and when R⁶ is heteroaryl, it is preferably pyridyl or quinol-2-yl; R² is preferably alkyl; and R³ is preferably hydrogen) are prepared as described in Reaction Scheme 2.

- 32 -

Reaction Scheme 2



SUBSTITUTE SHEET (RULE 28)

-33-

Starting Materials

N-protected amino acids of formula (A) and compounds of formula (B) are commercially available or may be prepared according to methods known to those of ordinary skill in the art. Compounds of formula (E) are prepared as described with reference to Reaction Scheme 1.

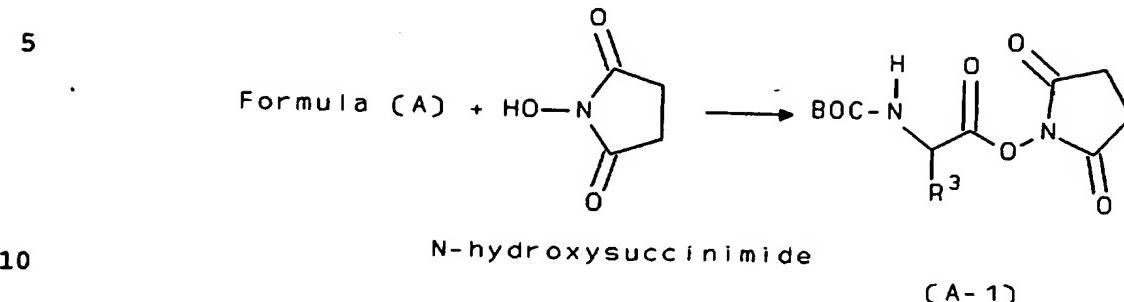
Formula (C) - In general, compounds of formula (Ia) are prepared by first coupling a compound of formula (A) with a compound of formula (B) [or with another compound of the formula $H_2N-(CH_2)_p-R^7$], under standard amide coupling conditions to form a compound of formula (C). For example, to a cold (0-5°C) solution of the compound of formula (A) and an excess molar amount of HOBT in DMF is added an excess molar amount of EDCI. The resulting solution is stirred from about 1 to about 2 hours, preferably for about 1 hour, at 0-5°C, preferably at 0°C. To the cold solution is then added a solution of an equimolar amount of a compound of formula (B) in the presence of a base, preferably DMAP. The resulting mixture is stirred from 12 to 24 hours, preferably for 24 hours, at room temperature, preferably at 25°C. The compound of formula (C) is then isolated from the reaction mixture by standard peptide isolation techniques.

Formula (D) - The amino-protecting group of the compound of formula (C) is then removed under mild acidic conditions, preferably in the presence of trifluoroacetic acid, to yield a compound of formula (D).

Alternative Preparations of Formula (D) - Another method of preparing a compound of formula (D) particularly when R³ is t-butyl, other β-branched amino acid side chains, or cyclohexyl, p is zero, and R⁷ is aryl or heteroaryl, employs the intermediate (A-1), the preparation of which is illustrated in Reaction Scheme 2A. Another alternative method of preparing a compound of formula (D) particularly when R₃ is 1-hydroxyisopropyl or another β-hydroxy amino acid side chain, and R⁷ is aryl or heteroaryl, is illustrated in Reaction Scheme 2B.

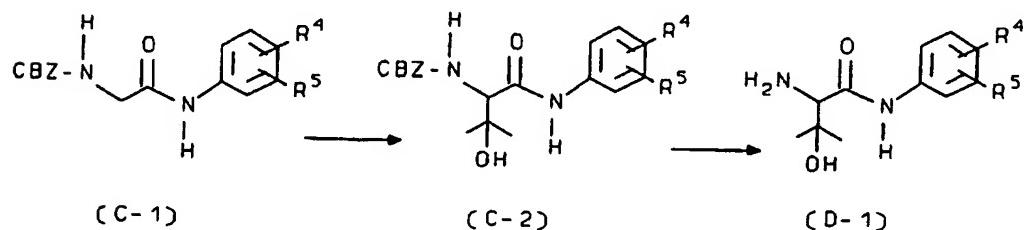
-34-

Reaction Scheme 2A



As illustrated in Reaction Scheme 2A, a compound of formula (A) is coupled with about one molar equivalent of N-hydroxysuccinimide in acetonitrile at 0°C in the presence of DCC. The reaction takes place with stirring at 0°C to 25°C, for 8 to 16 hours to give the corresponding N-hydroxysuccinimide ester of formula (A-1). This ester is then reacted with a compound of formula B or another compound of the formula $H_2N-(CH_2)_p-R^7$ in an inert solvent at 100°C preferably for 3 hours; the resultant compound of formula (C) is isolated and deprotected to yield a compound of formula (D) as described above in Reaction Scheme 2.

Reaction Scheme 2B



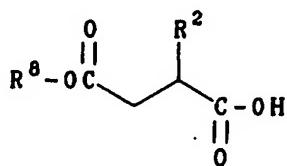
-35-

As illustrated in Reaction Scheme 2B, a compound of formula (C-1) in an inert anhydrous solvent such as THF is stirred with n-butyllithium at a temperature below 10°C, preferably 0°C, for about 1 hour, then cooled to about -70°C and reacted with 3 molar equivalents of acetone. A compound of formula (C-2), as a racemate, is isolated and purified by standard procedures. Following hydrogenolytic removal of the CBZ protecting group, a compound of formula (D-1) is obtained.

Formula (Ia) - As illustrated in Reaction Scheme 2, a compound of formula (D) is coupled with a compound of formula (E) under standard peptide coupling conditions. For example, to a cold (0-5°C, preferably 0°C) solution of the compound of formula (D) in an inert solvent, preferably THF, is added 1,1'-carbonyldiimidazole. The resulting mixture is stirred from 60 to 90 minutes, preferably for 75 minutes, at 0-5°C, preferably at 0°C, and then reacted with the compound of formula (E) for about 12 to 17 hours, preferably for about 15 hours. The resulting compound of formula (Ia) is then isolated from the reaction mixture by standard peptide isolation techniques, for example, extraction and reverse phase HPLC.

Preparation of Formula (F)

Compounds of formula (F) :

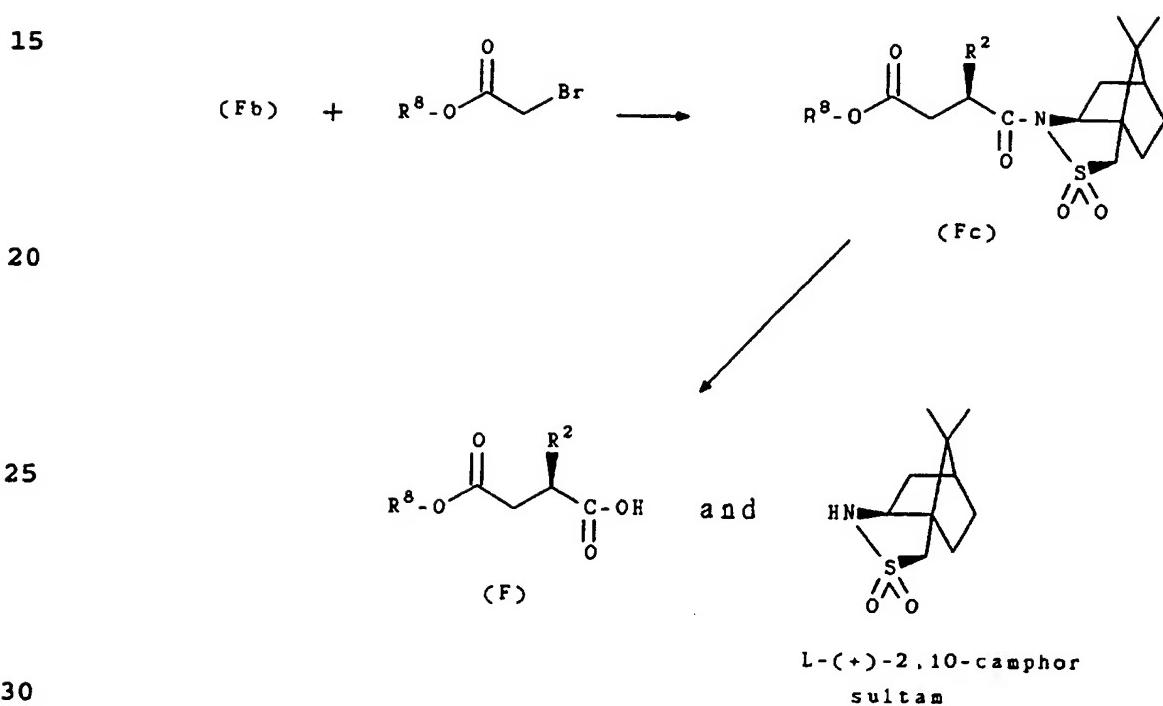
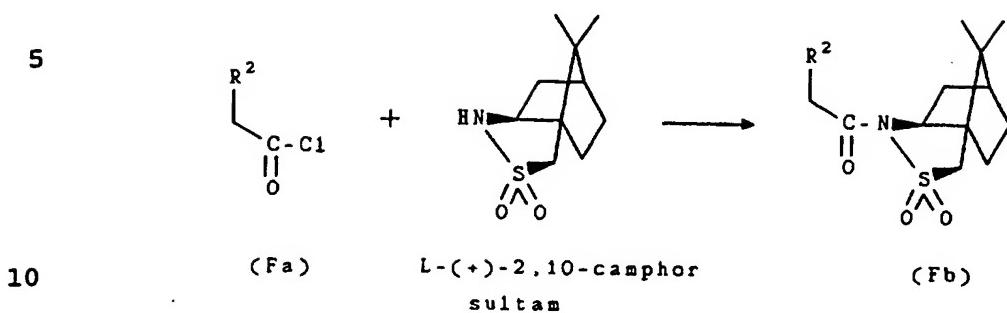


(F)

wherein R⁸ is t-butyl, are intermediates used in the preparation of compounds of formula (I) as illustrated below in Reaction Scheme 4. The compounds of formula (F) are prepared as shown in Reaction Scheme 3.

-36-

Reaction Scheme 3



Starting Materials

Compounds of formula (Fa) are commercially available or may be prepared according to methods known to those of ordinary skill in the art, for example, by the method described in Example 11 below. L-(+)-2,10-Camphor sultam and D-(-)-2,10-camphor sultam

-37-

are commercially available, for example, from Aldrich.

5 **Formula (Fb)** - In general, compounds of formula (F) (illustrated as one of the two isomers obtainable by this synthesis) are prepared by first condensing a compound of formula (Fa) [where the group R² encompasses the group "X" of Formula (I) and can be, e.g., a biphenylpropylene or fluorenyl propylene group] with L-(+)-2,10-camphor sultam to form a compound of formula (Fb).

10 **Formula (Fc)** - Using sodium hexamethyldisilazide to generate the anion for 1 hour, the reaction is quenched with t-butylbromoacetate to form the corresponding ester of formula (Fc).

15 **Formula (F)** - The camphor group is then removed under basic conditions, such as lithium hydroperoxide (formed *in situ* from lithium hydroxide and hydrogen peroxide) initially at reduced temperature (preferably 0°C) for 15 minutes and warmed to room temperature for 2 hours. The mixture is cooled back to 0°C and an aqueous mixture of sodium sulfite and sodium bicarbonate is added with stirring, after which the mixture is allowed to return to room temperature, and the pH is neutralized to yield an individual stereoisomer of a compound of formula (F) wherein the carbon to which the -X-R² group is attached is in the (R) configuration. In a similar manner, but substituting D-(-)-2,10-camphor sultam for L-(+)-2,10-camphor sultam, the corresponding individual stereoisomers in the (S) configuration can be prepared.

Alternative Preparation of Formula (F)

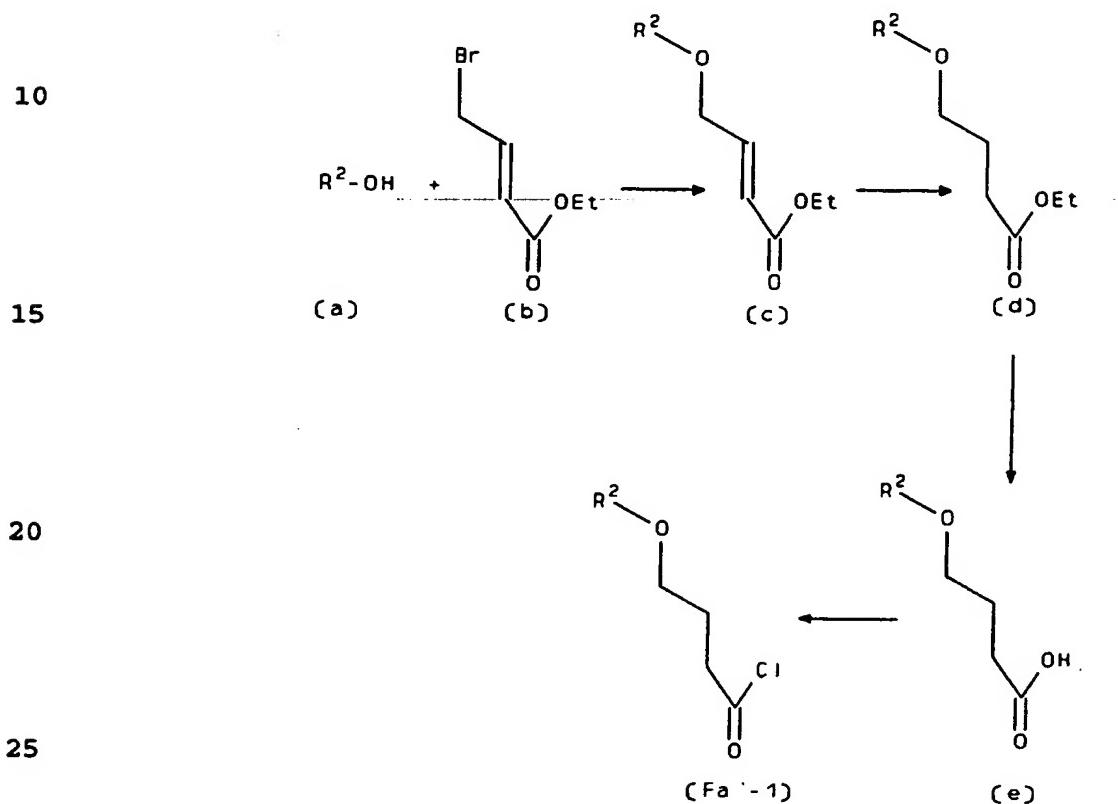
30 Another method of preparing stereoisomers of formula (F) utilizes the commercially available chiral compound, 4S-phenylmethylloxazolidinone, as shown below in Reaction Scheme 3A [following the Starting Materials section, where preparation of the compounds of starting material of formula (Fa') are illustrated].

-38-

Starting Materials

Compounds of formula (Fa') where X is $-O-CH_2-CH_2-$ are prepared as illustrated in Reaction Scheme 3A-1.

5

Reaction Scheme 3A-1

A commercially-available alcohol (a) is reacted with 30 ethyl-4-bromocrotonate (b) in the presence of stoichiometric sodium hydride in a solvent such as DMF at 0°C to room temperature, or in the case of a phenol (a), by refluxing with (b) in acetone in the presence of excess potassium carbonate for several hours. The resulting unsaturated ester (c) is 35 converted by hydrogenation in the presence of platinum on carbon to the saturated ester (d), which is then saponified with aqueous sodium hydroxide in ethanol to the acid (e). The

-39-

acid (e) is converted to the acid chloride (Fa'-1) through the action of oxalylchloride at between room temperature and 50°C.

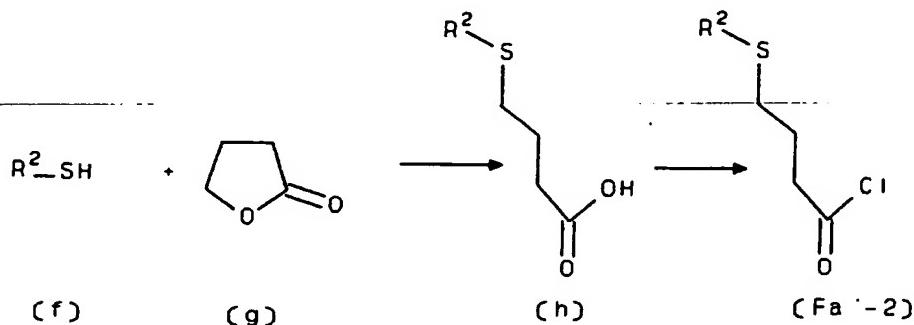
Compounds of formula (Fa') where X is -S-CH₂CH₂- are prepared as illustrated in Reaction Scheme 3A-2.

5

Reaction Scheme 3A-2

10

15



20

25

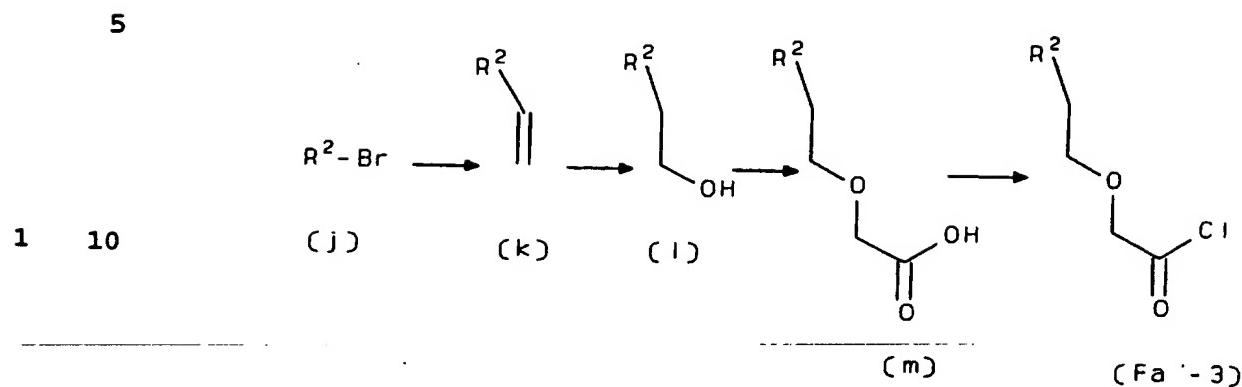
A commercially-available thiol (f) is reacted with lithium hydride in DMF at room temperature for several hours to form the lithium thiolate. Excess butyrolactone (g) is added and heated to reflux under argon to give the acid (h). Acid (h) is then converted to the acid chloride (Fa'-2) with oxalyl chloride, as before.

Compounds of formula (Fa') where X is -CH₂CH₂-O- are prepared as illustrated in Reaction Scheme 3A-3.

30

-40-

Reaction Scheme 3A-3



1 15

Compounds of formula (l) and (k) are in many cases commercially available. When not, they are prepared as follows. Compounds of formula (j), where R^2 is aryl or heteroaryl, are converted to alkenes (k) by treatment for several hours with vinyl-tributylstannane (commercially available from Aldrich Chemical Co.) in the presence of catalytic tetrakis (triphenylphosphine)palladium at reflux in toluene. The alkenes (k) may be further converted to the alcohols (l) by hydroboration with borane in THF at 0°C to room temperature, over a period of several hours, followed by oxidation with alkaline hydrogen peroxide. The alcohols (l) are converted to the acids (m) by treatment with chloroacetic acid and excess sodium hydride in DMF at elevated temperature, preferably 60°C. The acids (m) are converted to the acid chlorides (Fa'-3) with oxalyl chloride, as before.

Compounds of formula (Fa') where X is $-\text{CH}_2\text{CH}_2\text{-S-}$ are prepared according to Reaction Scheme 3A-4.

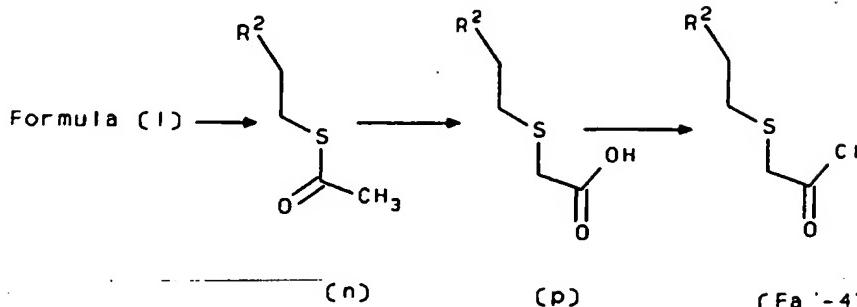
35

-41-

Reaction Scheme 3A-4

5

10



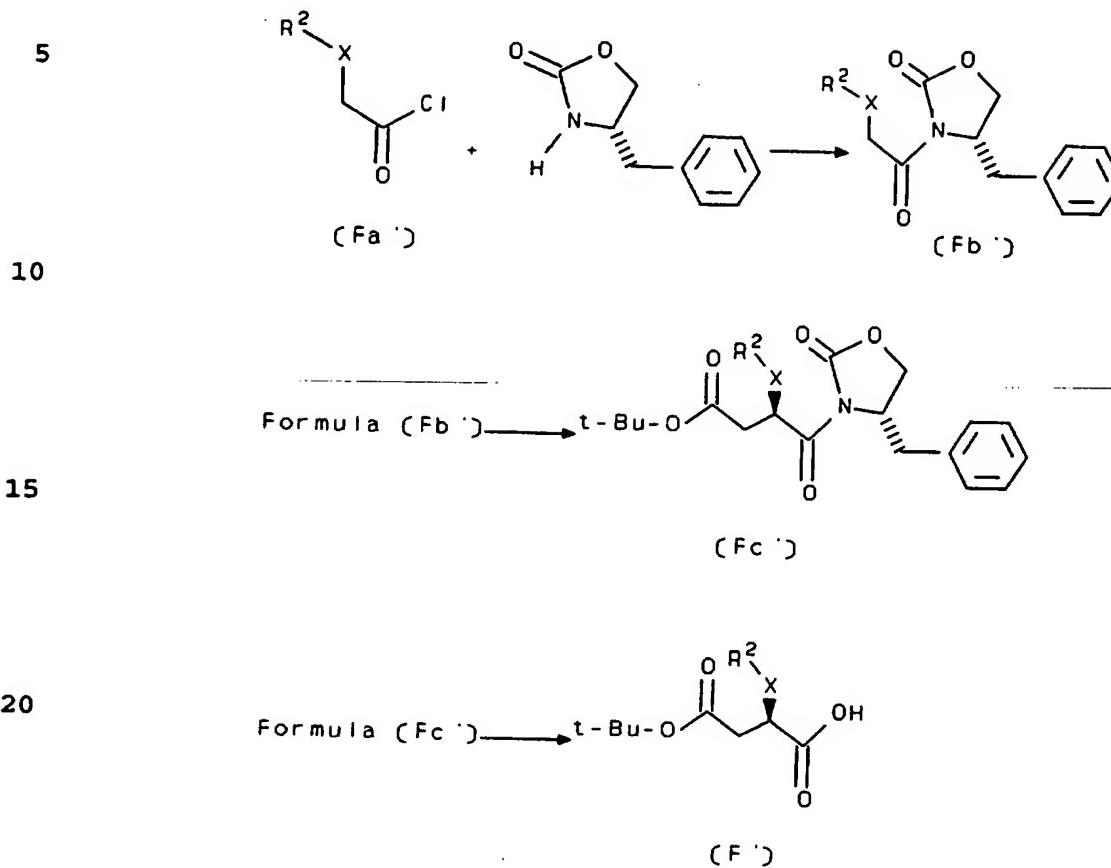
15

20

The alcohols (1) are converted to thioacetates (n) by addition of thioacetic acid to the reagent generated from triphosphine and diethyl azodicarboxylate in THF at 0°C. The thioacetates (n) are converted to the acids (p) by treatment with potassium carbonate in methanol in the presence of chloroacetic acid. The acids (p) are converted to the acid chloride (Fa'-4) with oxalyl chloride, as before.

25

Reaction Scheme 3A



Formula (Fb') - A compound of formula (Fa') is first condensed with 4S-phenylmethyloxazolidinone under standard conditions to give the corresponding compound of formula (Fb').

30 **Formula (Fc')** - An approximately equimolar amount of sodium hexamethyldisilazide is added to a compound of formula (Fb') in an inert solvent such as THF. The reaction takes place at -80°C to -95°C, for about 15 minutes.

35 t-Butylbromoacetate is added in excess to this mixture and the solution is stirred for about 2 hours at -90°C to -60°C to yield a single stereoisomer of formula (Fc'), which is purified by standard organic chemistry procedures.

-43-

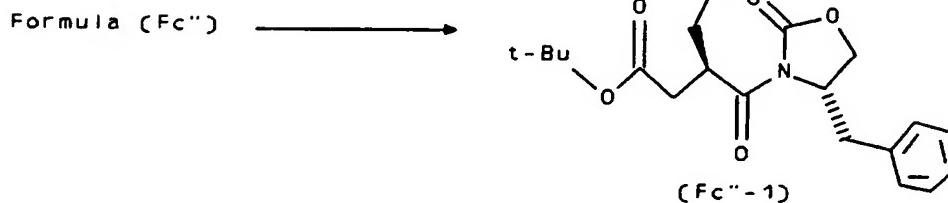
5 **Formula (F')** - The oxazolidinone group of a compound of formula (Fc') is removed under basic conditions to yield an individual stereoisomer of formula (F'), for example as described with reference to the preparation of formula (F) in Reaction Scheme 3. The compounds of formula (F') can be used interchangeably with those of formula (F) in the syntheses that follow.

10 **Alternative Preparation of Formula (F)** - Formula F can also be prepared as described with reference to Reaction Scheme 3B.

Reaction Scheme 3B

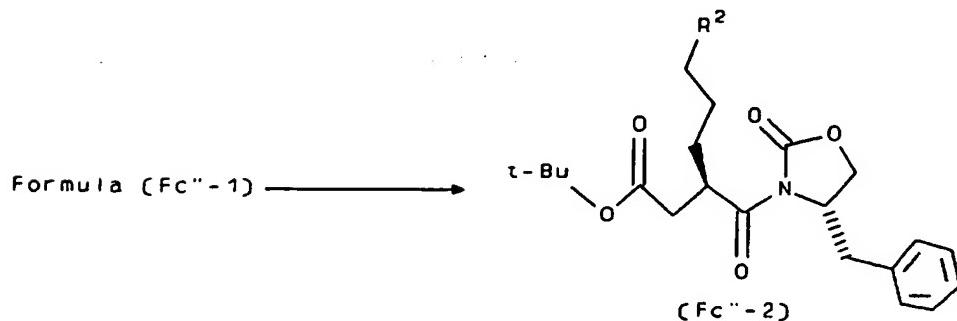
15

20



25

30



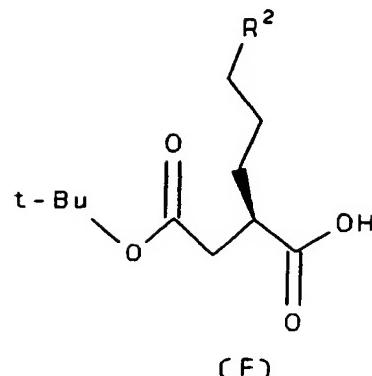
35

-44-

5

10

Formula (Fc"-2) →

**Starting Materials**

The compound illustrated as formula (Fc") can be prepared
15 analogously to the preparation of formula (Fc') as described
with reference to Reaction Scheme 3A, by substituting for the
compound of formula (Fa') the corresponding allyl compound
where the group shown as X is prop-2-enyl and R² is H.

20 Formula (Fc"-1) - Arylation or heteroarylation of (Fc") is carried out in the presence of a base and a palladium catalyst by adding aryl- or heteroaryl-halide, preferably bromide or iodide, and heating the reaction mixture for about 2 to 4 hours, preferably 4 hours, at about 100°C to form a compound of the formula (Fc"-1).

25 Formula (Fc"-2) - Catalytic hydrogenation (Pd/C) of an allyl compound of formula (Fc"-1) yields the corresponding alkyl compound of the formula (Fc"-2).

30 Formula (F) - A compound of formula (Fc"-2) is subjected to basic conditions, such as lithium hydroperoxide (formed in situ from lithium hydroxide and hydrogen peroxide) initially at reduced temperature (preferably 0°C) for 15 minutes and warmed to room temperature for 2 hours. The mixture is cooled back to 0°C and an aqueous mixture of sodium sulfite and sodium bicarbonate is added with stirring, after which the mixture is allowed to return to room temperature, the pH neutralized, and the compound of formula (F) is obtained by standard isolation.

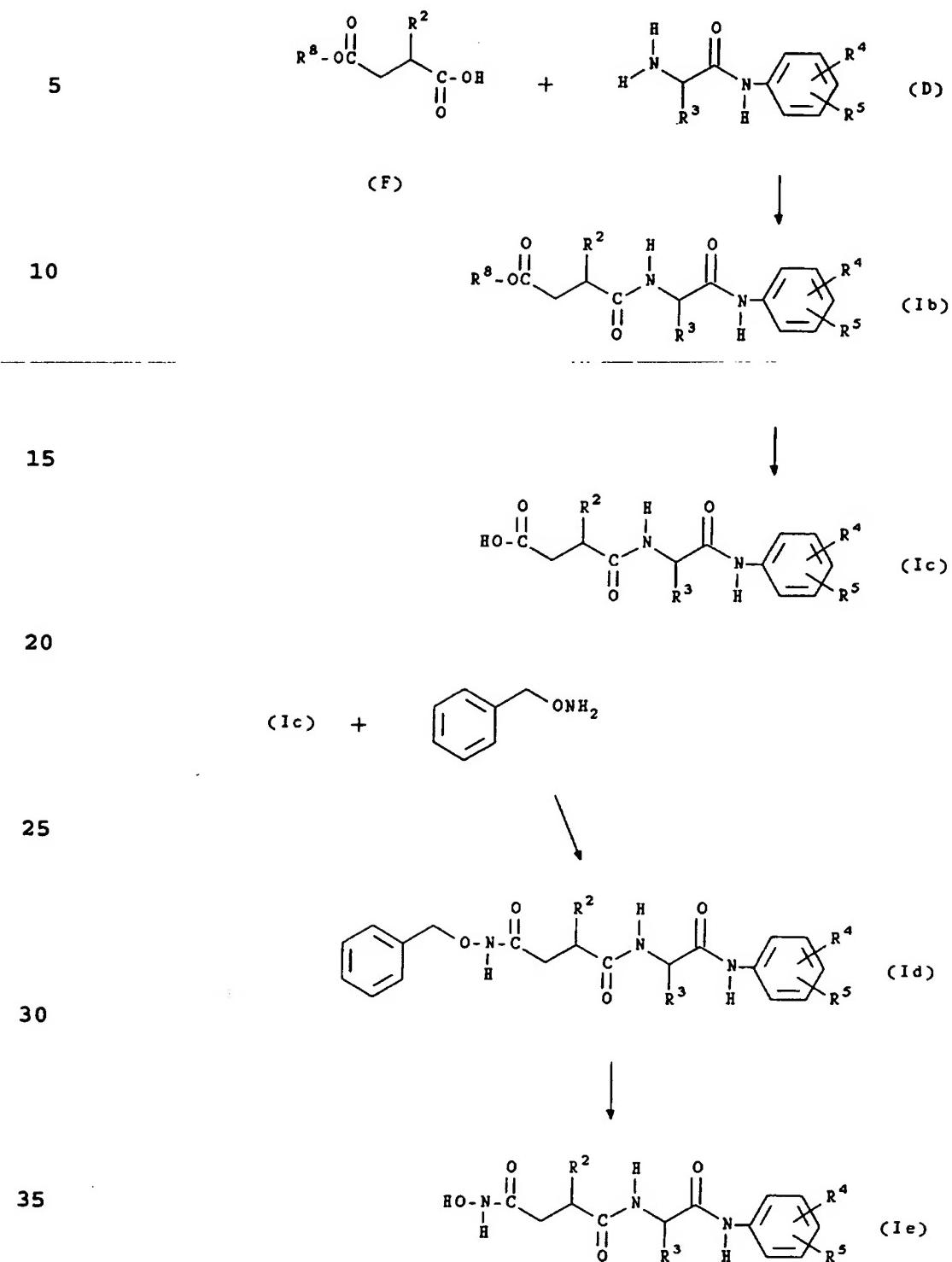
-45-

Preparation of Formulae (Ib), (Ic), (Id) and (Ie)

The compounds of formulae (Ib), (Ic), (Id) and (Ie) each represent sub-genuses of formula I in which the R¹ substituent varies, prepared sequentially as described in Reaction Scheme 4, where R⁸ is t-butyl. In compounds of formula (Ib) R¹ is alkoxy carbonyl or aralkoxy carbonyl. In compounds of formula (Ic) R¹ is carboxy. In compounds of formula (Id) R¹ is benzyloxycarbamoyl. In compounds of formula (Ie) R¹ is hydroxycarbamoyl.

-46-

Reaction Scheme 4



-47-

Starting Materials

Formula (D) is prepared as described with reference to Reaction Schemes 2, 2A and 2B. Formula (F) is prepared as described with reference to Reaction Schemes 3 and 3A.

5 O-Benzylhydroxylamine is commercially available, for example, as the hydrochloride salt from Aldrich Co.

Formula (Ib) - A compound of formula (F) is coupled with a compound of formula (D) under standard amide coupling conditions to form a compound of formula (Ib). For example, 10 to a solution of a compound of formula (F) in an aprotic solvent, preferably DMF, containing a slightly excess molar amount of HOBT, is added an excess molar amount of EDCI. The resulting mixture is stirred from 1 to 2 hours (preferably 1 hour) at 0-5°C (preferably at 0°C). To the cold solution is 15 then added an equimolar amount of a compound of formula (D) in the presence of a base, preferably DMAP. The resulting mixture is then stirred from 12 to 24 hours (preferably 24 hours) at room temperature (preferably at 25°C). The compound of formula (Ib) is then isolated from the reaction mixture by 20 standard peptide isolation techniques, for example, evaporation of solvents, extraction, flash chromatography and/or HPLC.

25 Formula (Ic) - A compound of formula (Ib) is hydrolyzed under mild acidic conditions, preferably with trifluoroacetic acid, to yield a compound of formula (Ic).

Formula (Id) - A compound of formula (Ic) is then treated with O-benzylhydroxylamine under standard amide coupling conditions to form a compound of formula (Id). For example, a cold (0-5°C) solution of the compound of formula (Ic) and HOBT 30 in an inert solvent, preferably DMF, is treated with an excess molar amount of EDCI. After stirring the resulting mixture for 30 minutes to an hour at 0-5°C (preferably at 0°C), an equimolar amount of O-benzylhydroxylamine is added. The reaction mixture is allowed to warm and remain at room 35 temperature overnight for 8 to 16 hours. The compound of formula (Id) is then isolated from the reaction mixture by standard isolation techniques, for example, by extraction and

-48-

flash chromatography.

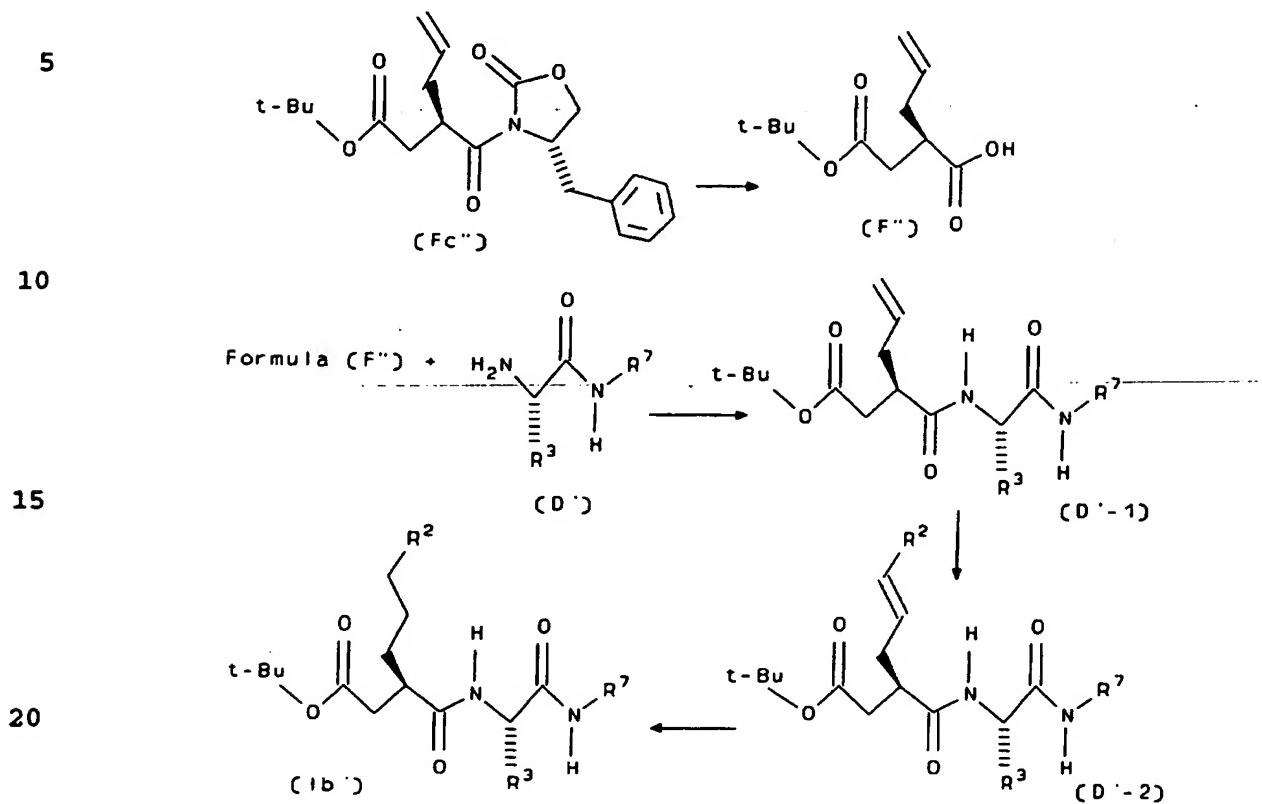
5 **Formula (Ie)** - The hydroxyl-protecting group (benzyl) of a compound of formula (Id) is removed under catalytic hydrogenation conditions (Pd/C) to yield a compound of formula (Ie).

10 **Alternative Preparation of Formula (Ie)** - An alternative method for preparing formula (Ie) (particularly where R⁴ is a sulfur-containing moiety, such as alkylsulfinyl) is to treat the corresponding compound of formula (Ic) with hydroxylamine hydrochloride and a peptide coupling reagent, preferably benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate, in the presence of a tertiary amine base such as N-methylmorpholine in DMF solvent. The resulting compound of formula (Ie) is isolated from the reaction mixture by standard isolation techniques, for example, by extraction and concentration.

15 **Alternative Preparation of Formula (Ib)** - A particularly preferred method of preparing compounds of formula (Ib) when R² is an aryl or heteroaryl, and where X (not shown) is propane-1,3-diyl and p (not shown) is zero is shown in Reaction Scheme 20 4A.

-49-

Reaction Scheme 4A



25 Starting Materials

The compound illustrated as formula (Fc") can be prepared analogously to the preparation of formula (Fc') as described with reference to Reaction Scheme 3A, by substituting for the compound of formula (Fa') the corresponding allyl compound where R² is prop-2-enyl. The compound of formula (D') is a compound of formula (D) and can be as described in Reaction Scheme 2. The halo-aryl or halo-heteroaryl reactants used in the preparation of compounds of formula (D'-2) are commercially available, or can be prepared according to methods known to those of ordinary skill in the art, e.g., as described in Example 41C.

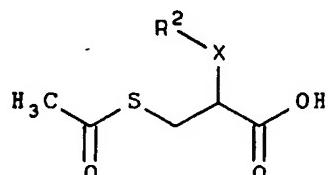
-50-

A compound of formula (F'') is prepared by alkaline hydrolysis of the oxazolidinone group from a compound of formula (Fc''). After isolation by standard procedures, (F'') is coupled with a compound of formula (D') under standard peptide coupling conditions as described above with reference to Reaction Scheme 2, to form a compound of formula (D'-1).
 5 Arylation or heteroarylation of (D'-1) is accomplished by adding aryl- or heteroaryl halide (preferably aryl- or heteroaryl bromide, iodide or triflate) and heating the reaction mixture for about 2 hours at about 100°C to form a compound of the formula (D'-2). Catalytic hydrogenation
 10 (Pd/C) of (D'-2) yields a compound of formula (Ib').

15 Preparation of Formula (G)

Compounds of formula (G) :

20



25

(G)

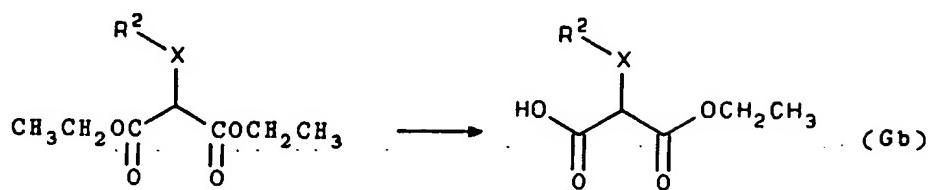
30

are intermediates in the preparation of compounds of formula (I) and are prepared as illustrated below in Reaction Scheme 6. The compounds of formula (G) are prepared as shown in Reaction Scheme 5.

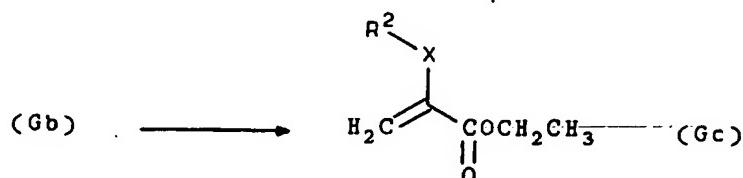
35

-51-

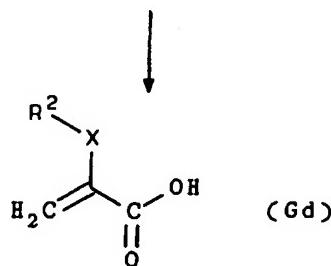
Reaction Schemes 5



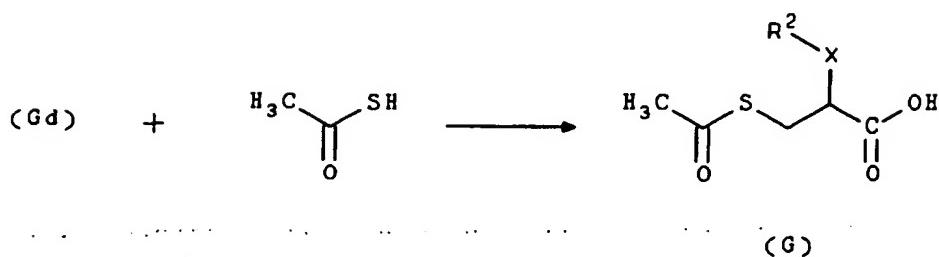
(Ga)



15



20



30

Starting Materials

Compounds of formula (Ga) and thioacetic acid are commercially available, for example, from TCI America Organic Chemicals and the Aldrich Company, respectively.

35

Formula (Gb) - A compound of formula (Ga) is hydrolyzed with an equimolar amount of a base, for example, potassium

-52-

hydroxide, to yield a compound of formula (Gb).

Formula (Gc) - A compound of formula (Gb) is deprotonated under basic conditions, for example, in the presence of triethylamine, at 0-5°C (preferably at 0°C) and then reacted with formaldehyde, followed by treatment with aqueous base, preferably potassium carbonate, to yield a compound of formula (Gc), which is isolated from the reaction mixture by standard isolation procedures.

Formula (Gd) - A compound of formula (Gc) is hydrolyzed under basic conditions, preferably in the presence of lithium hydroxide, to yield a compound of formula (Gd).

Formula (G) - A compound of formula (Gd) is reacted with an excess molar amount of thioacetic acid at 90-100°C (preferably at 95°C) under an inert atmosphere. The compound of formula (G) is then isolated from the reaction mixture by standard isolation techniques, for example, by extraction and evaporation.

Preparation of Formulae (If) and (Ig)

The compounds of formulae (If) and (Ig) each represent sub-genuses of formula I in which the R¹ substituent is sulfur-containing, prepared sequentially as described in Reaction Scheme 6. In compounds of formula (If), R¹ is acetylthio. In compounds of formula (Ig), R¹ is mercapto.

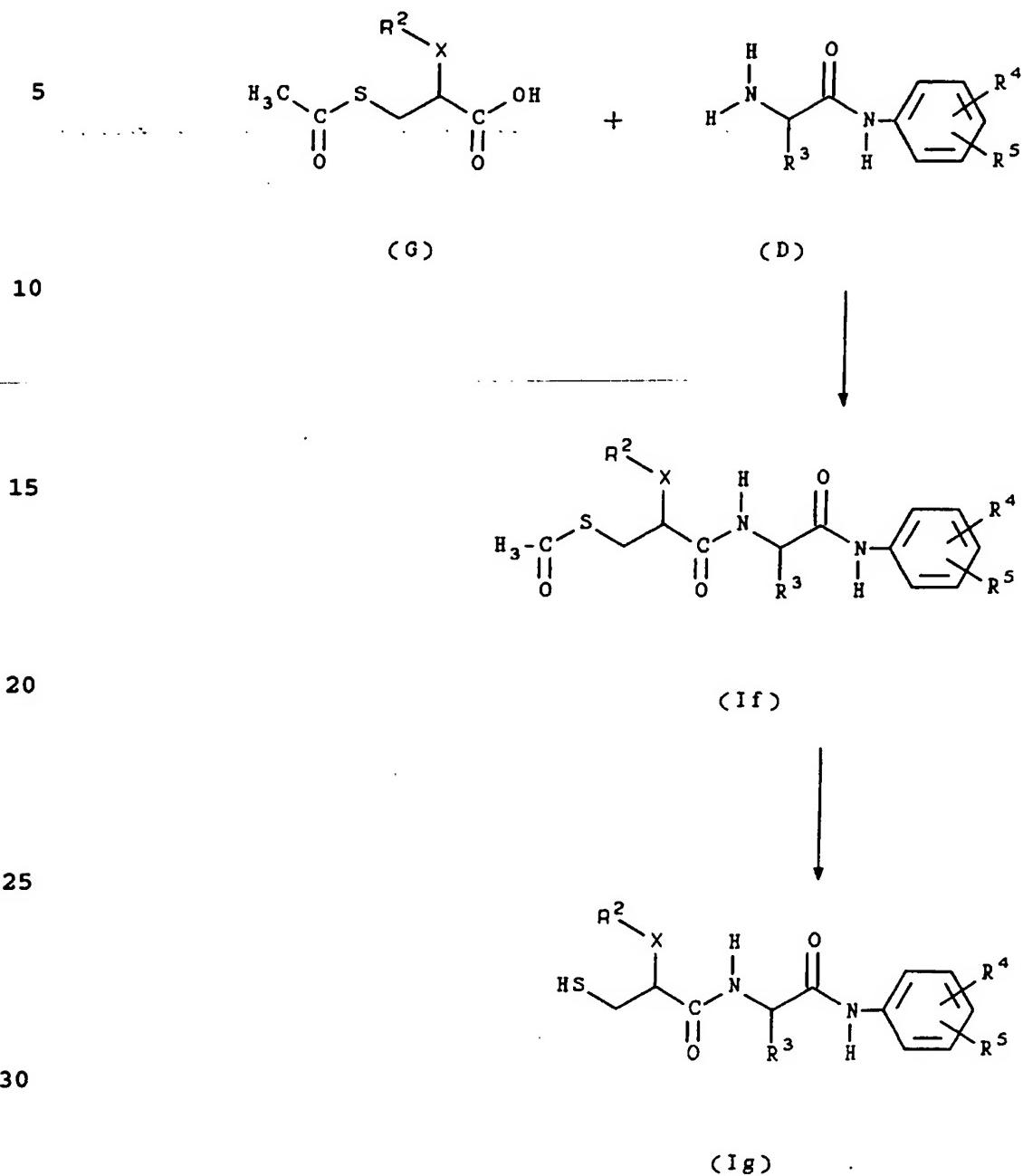
25

30

35

-53-

Reaction Scheme 6



35 Formula (If)

A compound of formula (G) is coupled with a compound of formula (D) under standard amide coupling conditions to yield

-54-

a compound of formula (If). For example, to a solution of the compound of formula (G) and HOBT in an aprotic solvent, preferably DMF, is added an excess molar amount of EDCI. Subsequently, the compound of formula (D) is added and the resulting mixture is stirred overnight at room temperature.

5 The resulting compound of formula (If) is then isolated from the reaction mixture by standard isolation techniques, for example, by evaporation of solvent, extraction, and flash chromatography.

10 Formula (Ig) - A compound of formula (If) is hydrolyzed under basic conditions, preferably in a protic solvent such as methanol in the presence of ammonium hydroxide, to form a compound of formula (Ig).

15 **Preparation of Formula (Ih)**

Compounds of formula (Ih) are a sub-genus of formula (I) where R¹ is N-hydroxyformylamino, and are prepared as shown in Reaction Scheme 7.

20

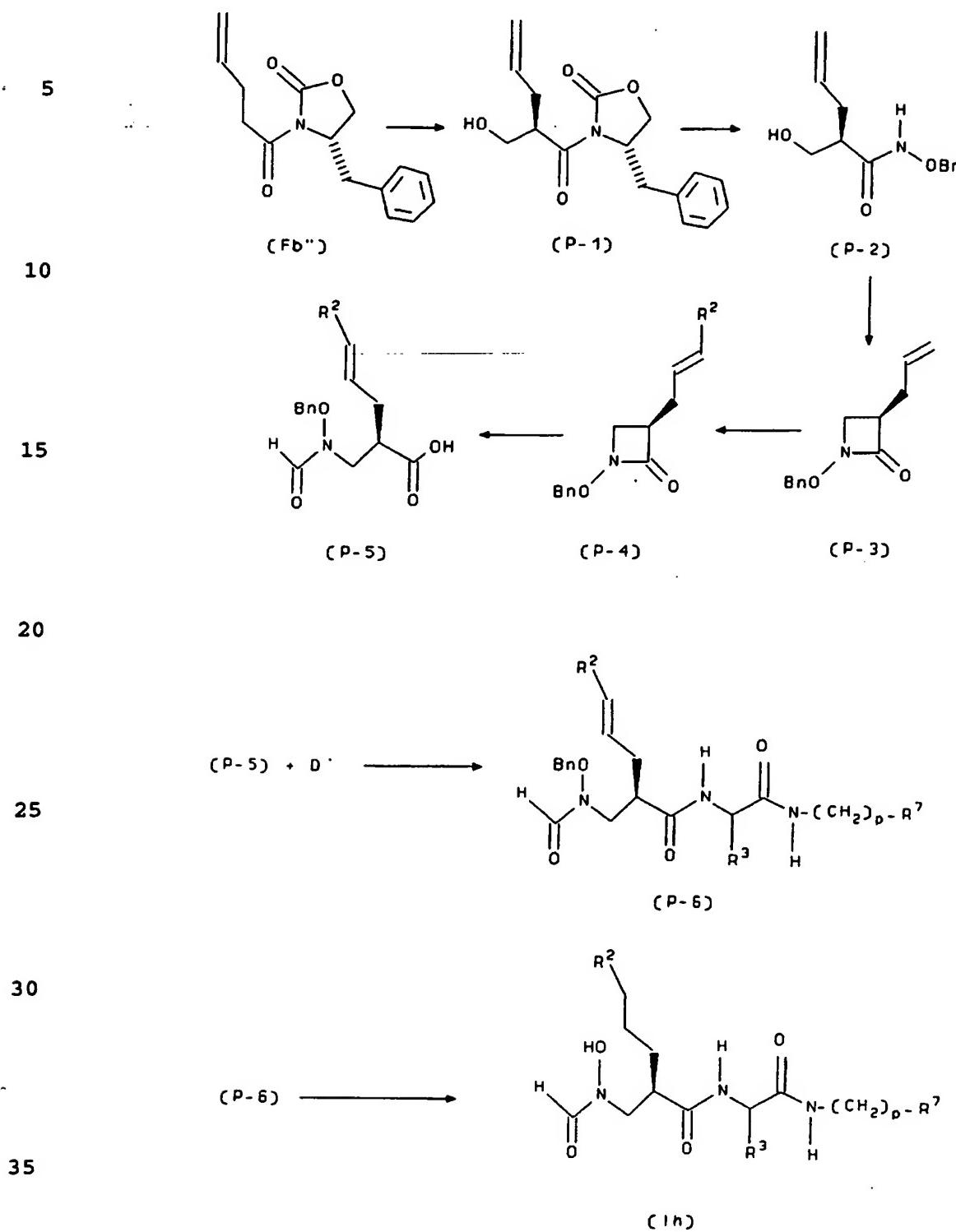
25

30

35

-55-

Reaction Scheme 7



-56-

Starting Materials

The compound illustrated as formula (Fb") can be prepared analogously to the preparation of formula (Fb') as described with reference to Reaction Scheme 3A, by substituting for the 5 compound of formula (Fa') the corresponding allyl compound where R² is prop-2-enyl.

Formula (P-1) - The compound of formula (Fb") is hydroxymethylated by incubation with titanium tetrachloride at reduced temperature, preferably 0°C, under basic conditions 10 for one to three hours, preferably 1 hour followed by addition of S-trioxane and titanium tetrachloride with continued incubation at 0°C for 3 to 5 hours, preferably 4 hours. The compound of formula (P-1) is then isolated by standard methods, e.g., extraction and column chromatography.

Formula (P-2) - The compound of formula (P-1) is reacted with an excess molar amount of 0-benzylhydroxylamine and of trimethylaluminum at reduced temperature, preferably 0°C. The reaction is allowed to proceed with stirring for 5 to 7 hours, preferably 6 hours, at 0°C under argon. The resulting 20 compound of formula (P-2) is isolated by standard procedures.

Formula (P-3) - Excess mesyl chloride is reacted with the compound of formula (P-2) in pyridine at 0°C for several hours, preferably 3 hours. The reaction mixture is cooled on ice, organic solvent-extracted, and concentrated. The 25 concentrated extract is refluxed under basic conditions for several hours, preferably 3 hours, thus yielding the azetidinone compound of formula (P-3), which is purified by standard procedures.

Formula (P-4) - The compound of formula (P-3) is reacted with a desired halogenated R² group (e.g., an aryl- or heteroaryl halide, preferably bromide or iodide) in an inert solvent in the presence of a base, such as triethylamine, and a palladium catalyst, preferably formed from palladium (II) acetate and about 2 molar equivalents of tri-o-tolylphosphine. After heating the reaction mixture for 15 to 20 hours, 35 preferably 18 hours at 100°C, the corresponding compound of formula (P-4) is isolated and purified by standard procedures.

5 **Formula (P-5)** - Cleavage of the azetidinone ring of a compound of formula (P-4) is carried out under basic conditions at room temperature for 1 to 3 hours, preferably 1 hour. The resultant compound is extracted into organic solvent, concentrated, redissolved in a base-containing solvent (e.g., pyridine), and carboxylated with formic anhydride at reduced temperature, preferably 0°C, for 30 minutes, to yield the corresponding compound of formula (P-5),
10 which is isolated by standard procedures.

15 **Formula (P-6)** - A compound of formula (P-5) is coupled with a compound of formula (D') under standard amide coupling conditions to form the corresponding compound of formula (P-6), which is isolated by standard procedures.

20 **Formula (Ih)** - Catalytic hydrogenation of a compound of formula (P-6) with Pd/C, followed by removal of the catalyst by filtration yields the corresponding compound of formula (Ih).

25 **Preparation of Salts**

In addition, all compounds of formula (I) that exist in either the free acid or the free base form may be converted to their pharmaceutically acceptable salts by treatment with the appropriate inorganic or organic base or with the appropriate inorganic or organic acid, respectively. Salts of the compounds of formula (I) can also be converted to the free acid or free base form or to another salt. For example, a compound of formula (I) having a carboxylic acid moiety can be converted to the carboxylate form by addition of 1 equivalent of NaOH or KOH in an alcoholic solvent followed by evaporation of solvent. A compound of formula (I) in the form of a free base can be converted to the chloride salt, for example, by addition of 1 equivalent of HCl in an organic solvent, followed by concentration.

30

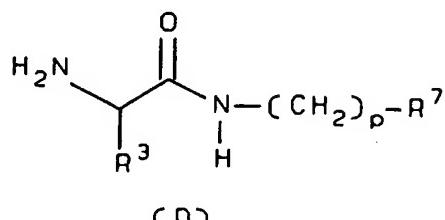
35 **Preferred Synthesis and Last Steps**

In summary, compounds of formula (I) are prepared by:

-58-

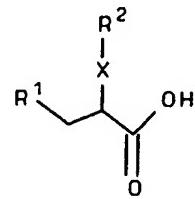
(A) contacting a compound of formula (D)

5



10 with a compound of formula (F)

15

20 wherein R¹ is alkoxy carbonyl, aralkoxy carbonyl, aryl- or heteroaryl thiomethylphosphinoyl, or acetylthio;

in the presence of a base and an amide coupling reagent to give the corresponding compound of formula (I); or

25 (B) catalytically hydrogenating the corresponding compound where X and R² together are optionally aryl- or heteroaryl-substituted alkenyl; or(C) treating a compound of formula (I), where R¹ is alkoxy carbonyl or aralkoxy carbonyl, under mild acidic conditions to give the corresponding compound of formula (I) where R¹ is carboxy; or(D) contacting a compound of formula (I), where R¹ is carboxy, with O-benzylhydroxylamine to give the corresponding compound of formula (I) where R¹ is benzyloxycarbamoyl; or30 (E) catalytically hydrogenating a compound of formula (I), where R¹ is benzyloxycarbamoyl, to give the corresponding compound of formula (I) where R¹ is hydroxycarbamoyl; or(F) contacting a compound of formula (I), where R¹ is

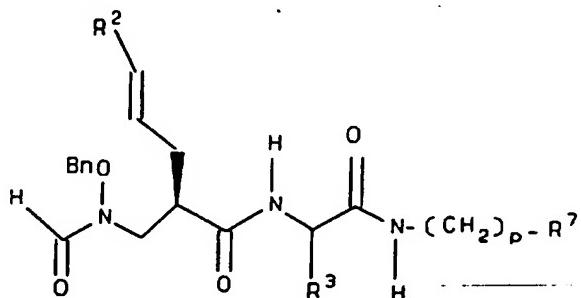
-59-

carboxy, with hydroxylamine to give the corresponding compound of formula (I) where R¹ is hydroxycarbamoyl; or

(G) catalytically hydrogenating a compound of the formula

5

10



15 wherein BnO is benzyloxy, to give the corresponding compound of formula (I) where R¹ is N-hydroxyformylamino; or

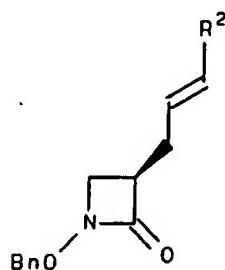
20

(H) treating a compound of formula (I), wherein R¹ is acetylthio, with ammonium hydroxide in a protic solvent to give the corresponding compound of formula (I) where R¹ is mercapto.

25

A preferred method of making compounds of formula (I) where R¹ is N-hydroxyformylamino entails converting a compound of formula (P-4)

30



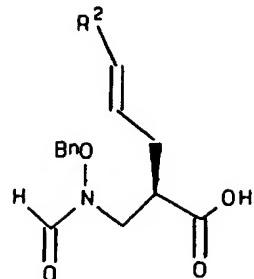
(P-4)

35

wherein R² is aryl or heteroaryl, by basic hydrolysis followed by formylation to give a compound of formula (P-5)

-60-

5



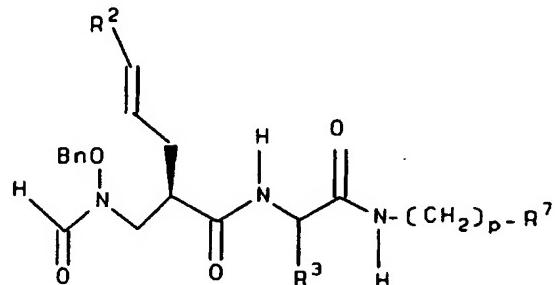
10

(P-5)

reacting the compound of formula (P-5) with a compound of formula (D) to give a compound of formula (P-6)

15

20



(P-6)

25

and catalytically hydrogenating the compound of formula (P-6).

30

35

Compounds prepared by the above-described process of the invention may be identified by the presence of a detectable amount of one or more compounds of formulae (P-3), (P-4) or (P-6). While it is well known that pharmaceuticals must meet pharmacopoeia standards before approval and/or marketing, and that synthetic reagents (such as O-benzylhydroxylamine) or precursors [such as (P-3), (P-4), or (P-6)] should not exceed the limits prescribed by pharmacopoeia standards, final compounds prepared by a process of the present invention may have minor, but detectable, amounts of such materials present, for example at levels in the range of 50ppm or lower. These

-61-

levels of (P-3) can be detected, e.g., by GC-MS, or of (P-4) can be detected, e.g., by HPLC-MS or by HPLC with fluorescence detection, or of (P-6) can be detected, e.g., by HPLC with fluorescence detection. It is important to monitor the purity 5 of pharmaceutical compounds for the presence of such materials, which presence is additionally disclosed as a method of detecting use of a process of the invention.

10

EXAMPLES

15

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

Example 1

Compounds of formula (Ea)

1A. Crystalline phosphinic acid (8.4 g, 0.13 mol) was stirred 20 in neat triethylorthoformate (22 mL, 0.20 mL) for 90 minutes at room temperature. This was then transferred via cannula to a stirred solution of ethylisobutylacrylate (8 g, 0.036 mol) and tetramethylguanidine (4.5 mL, 0.036 mol) that had been cooled to 0°C for 10 minutes. The ice bath was removed and 25 the reaction stirred for 4 hours. The mixture was diluted with 200 mL of diethyl ether and the solution washed with 1 N HCl (100 mL), water (4x100 mL) brine (100 mL), and dried over magnesium sulfate. This was rotary-evaporated to yield 8.15 g of 2-(ethoxy)phosphinoylmethyl-4-methylpentanoic acid ethyl ester as a slightly yellow colored oil, MS: 349 ($M - H_2O$)⁺.

30

1B. In a similar manner, the following compounds of formula (Ea) are prepared:

35

2-(ethoxy)phosphinoylmethyl-5-phenylpentanoic acid ethyl ester;

2-(ethoxy)phosphinoylmethyl-4-phenylbutanoic acid ethyl ester;

2-(ethoxy)phosphinoylmethyl-3-phenylpropanoic acid ethyl ester;

-62-

2-(ethoxy)phosphinylmethyl-3-cyclohexylpropanoic acid ethyl ester; and

2-((ethoxy)phosphinylmethyl)pentanoic acid ethyl ester.

5

Example 2

Compound of formula (Eb)

2A. Crude 2-(ethoxy)phosphinylmethyl-4-methylpentanoic acid ethyl ester (26 g) was dissolved in 600 mL THF/CH₂Cl₂ (50/50) and cooled to 0°C. Diisopropylethylamine (32 mL) and 90.8 mL of bis(trimethylsilyl) acetamide were then added to the solution and the resulting mixture was stirred for 20 minutes before paraformaldehyde (5.5 g) was added. The solution was brought to room temperature and heated at 37°C for 18 hours. The solvent was removed by evaporation, and the resulting oil dissolved in 200 mL ethyl acetate. The solution was washed with 50 mL of 1N HCl (2x), 50 mL of brine (2x), dried over MgSO₄, filtered and evaporated to yield 19.3 g of 2-(ethoxy)(hydroxymethyl)phosphinylmethyl-4-methylpentanoic acid ethyl ester as a faintly yellow oil, MS: 281.2 (MH⁺).

2B. In a similar manner, the following compounds of formula (Eb) are prepared:

2-(ethoxy)(hydroxymethyl)phosphinylmethyl-5-phenylpentanoic acid ethyl ester;

2-(ethoxy)(hydroxymethyl)phosphinylmethyl-4-phenylbutanoic acid ethyl ester;

2-(ethoxy)(hydroxymethyl)phosphinylmethyl-3-phenylpropanoic acid ethyl ester;

2-(ethoxy)(hydroxymethyl)phosphinylmethyl-3-cyclohexylpropanoic acid ethyl ester; and

2-((ethoxy)(hydroxymethyl)phosphinylmethyl)pentanoic acid ethyl ester.

Example 3

Compounds of formula (Ec)

3A. 2-(Ethoxy)(hydroxymethyl)phosphinylmethyl-4-methylpentanoic acid ethyl ester (5 g) was dissolved in 20 mL of CH₂Cl₂ and cooled to -20°C (in duplicate). Methanesulfonyl

-63-

chloride (1.5 mL) and triethylamine (3.0 mL) were added to solution dropwise. After 15 minutes the bath was removed and the reaction left at room temperature for 3½ hours. Each solution was then washed with 10 mL cold 2% HCl, 10 mL NaHCO₃ (sat), 10 mL brine, dried with MgSO₄, filtered and evaporated to yield 12.8 g (combined yield) of 2-(ethoxy)(methane-sulfonyloxy)methyl phosphinoylmethyl-4-methylpentanoic acid ethyl ester.

10 3B. In a similar manner, but replacing methanesulfonyl chloride with *p*-toluenesulfonyl chloride, 2-(ethoxy)-(*p*-toluenesulfonyloxy)methyl phosphinoylmethyl-4-methylpentanoic acid ethyl ester is prepared.

15 3C. In a similar manner, the following compounds of formula (Ec) are prepared:

2-(ethoxy)(methanesulfonyloxy)methyl phosphinoylmethyl-5-phenylpentanoic acid ethyl ester;

2-(ethoxy)(methanesulfonyloxy)methyl phosphinoylmethyl-4-phenylbutanoic acid ethyl ester;

2-(ethoxy)(methanesulfonyloxy)methyl phosphinoylmethyl-3-phenylpropanoic acid ethyl ester;

2-(ethoxy)(methanesulfonyloxy)methyl phosphinoylmethyl-3-cyclohexylpropanoic acid ethyl ester;

25 2-((ethoxy)(methanesulfonyloxy)methyl)phosphinoylmethyl-5-pentanoic acid ethyl ester;

2-(ethoxy)(*p*-toluenesulfonyloxy)methyl phosphinoylmethyl-5-phenylpentanoic acid ethyl ester;

2-(ethoxy)(*p*-toluenesulfonyloxy)methyl phosphinoylmethyl-4-phenylbutanoic acid ethyl ester;

2-(ethoxy)(*p*-toluenesulfonyloxy)methyl phosphinoylmethyl-3-phenylpropanoic acid ethyl ester;

2-(ethoxy)(*p*-toluenesulfonyloxy)methyl phosphinoylmethyl-3-cyclohexylpropanoic acid ethyl ester; and

35 2-((ethoxy)(*p*-toluenesulfonyloxy)methyl)phosphinoylmethyl-5-pentanoic acid ethyl ester.

-64-

Example 4

Compounds of formula (Ee)

- 4A. Sodium hydride (1.52 g, (60%)) and 2-quinolinethiol (6 g) were stirred together at 0°C in 50 mL DMF. After the initial H₂ evolution had subsided, the mixture was stirred at room temperature for 2.5 hours. The mixture was then cooled to 0°C and 2-(ethoxy)(methanesulfonyloxyethyl)phosphinoylmethyl-4-methylpentanoic acid ethyl ester (12.8 g) in 10 mL DMF was added via cannula and the resulting mixture was then stirred for 18 hours, slowly warming to room temperature. The DMF was removed by evaporation, the residue dissolved in 50 mL ethyl acetate and washed with 50 mL H₂O (2x), brine (50 mL), dried with MgSO₄, and evaporated to a yellow semi-solid. Purification by flash chromatography using 10% ethyl acetate/hexane to 80% ethyl acetate/hexane for the elution yielded 10 g of 2-(ethoxy)(quinolin-2-ylthiomethyl)phosphinoyl-methyl-4-methylpentanoic acid ethyl ester (Rf 0.35 80% ethyl acetate/hexane), MS: 424.1 (MH⁺).
- 4B. In a similar manner, but replacing 2-quinolinethiol with 1-naphthalenethiol, 2-naphthalenethiol or thiophenol, the following compounds of formula (Ee) are prepared:
2-(ethoxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-4-methylpentanoic acid ethyl ester;
2-(ethoxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoic acid ethyl ester; and
2-(ethoxy)(phenylthiomethyl)phosphinoylmethyl-4-methylpentanoic acid ethyl ester.
- 4C. In a similar manner, the following compounds of formula (Ee) are prepared:
2-(ethoxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-5-phenylpentanoic acid ethyl ester;
2-(ethoxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-phenylbutanoic acid ethyl ester;
2-(ethoxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-3-phenylpropanoic acid ethyl ester;

-65-

- 2-(ethoxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-
3-cyclohexylpropanoic acid ethyl ester;
2-((ethoxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl)-
pentanoic acid ethyl ester;
- 5 2-(ethoxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
5-phenylpentanoic acid ethyl ester;
2-(ethoxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
4-phenylbutanoic acid ethyl ester;
2-(ethoxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
10 3-phenylpropanoic acid ethyl ester;
2-(ethoxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
3-cyclohexylpropanoic acid ethyl ester;
2-((ethoxy)(naphth-1-ylthiomethyl)phosphinoylmethyl)-
pentanoic acid ethyl ester;
- 15 2-(ethoxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
5-phenylpentanoic acid ethyl ester;
2-(ethoxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
4-phenylbutanoic acid ethyl ester;
2-(ethoxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
20 3-phenylpropanoic acid ethyl ester;
2-(ethoxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
3-cyclohexylpropanoic acid ethyl ester;
2-((ethoxy)(naphth-2-ylthiomethyl)phosphinoylmethyl)-
pentanoic acid ethyl ester;
- 25 2-(ethoxy)(phenylthiomethyl)phosphinoylmethyl-
5-phenylpentanoic acid ethyl ester;
2-(ethoxy)(phenylthiomethyl)phosphinoylmethyl-
4-phenylbutanoic acid ethyl ester;
2-(ethoxy)(phenylthiomethyl)phosphinoylmethyl-
30 3-phenylpropanoic acid ethyl ester;
2-(ethoxy)(phenylthiomethyl)phosphinoylmethyl-
3-cyclohexylpropanoic acid ethyl ester; and
2-((ethoxy)(phenylthiomethyl)phosphinoylmethyl)-
pentanoic acid ethyl ester.

-66-

Example 5

Compounds of formula (E)

5 **5A.** 2-(Ethoxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoic acid ethyl ester (4.5 g) was dissolved in 100 mL THF and 12.5 mL of 2N NaOH was added together with enough methanol to make the solution homogeneous. After 18 hours the THF was removed by evaporation, the residue diluted with 50 mL H₂O and washed with 50 mL ethyl acetate. The aqueous phase was then acidified to pH 4, and the product extracted with 50 mL ethyl acetate (2x). The ethyl acetate was washed with 20 mL brine, dried with MgSO₄, and evaporated to yield 3.8 g of 2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoic acid as a yellow oil, MS: 368 (MH⁺).

15 **5B.** In a similar manner, the following compounds of formula (E) are prepared:

2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-4-methylpentanoic acid;

2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoic acid; and

2-(hydroxy)(phenylthiomethyl)phosphinoylmethyl-4-methylpentanoic acid.

25 **5C.** In a similar manner, the following compounds of formula (E) are prepared:

2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-5-phenylpentanoic acid;

2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-phenylbutanoic acid;

30 2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-3-phenylpropanoic acid;

2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-3-cyclohexylpropanoic acid;

35 2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-pentanoic acid;

2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-5-phenylpentanoic acid;

-67-

- 2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
4-phenylbutanoic acid;
2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
3-phenylpropanoic acid;
5 2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
3-cyclohexylpropanoic acid;
2-((hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl)-
pentanoic acid;
2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
10 5-phenylpentanoic acid;
2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
4-phenylbutanoic acid;
2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
3-phenylpropanoic acid;
15 2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
3-cyclohexylpropanoic acid;
2-((hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl)-
pentanoic acid;
2-(hydroxy)(phenylthiomethyl)phosphinoylmethyl-
20 5-phenylpentanoic acid;
2-(hydroxy)(phenylthiomethyl)phosphinoylmethyl-
4-phenylbutanoic acid;
2-(hydroxy)(phenylthiomethyl)phosphinoylmethyl-
3-phenylpropanoic acid;
25 2-(hydroxy)(phenylthiomethyl)phosphinoylmethyl-
3-cyclohexylpropanoic acid; and
2-((hydroxy)(phenylthiomethyl)phosphinoylmethyl)pentanoic
acid.

30

Example 6

Resolution of a compound of formula (E)

2-(Hydroxy)(quinolin-2-ylthiomethyl)phosphinoyl-methyl-4-methylpentanoic acid (5.3 g) was dissolved in 50 mL of warm ethanol (abs) and 4.2 g of (-)-cinchonidine was added. After 30 minutes at room temperature the salt began to precipitate out. The flask was covered in foil and allowed to stand for 2 days. The salt was then removed by suction filtration, and

-68-

the filtrate evaporated to a yellow foam. The salt and the filtrate were each dissolved in 100 mL ethyl acetate and washed successively with 1% HCl to remove the cinchonidine while keeping the pH above 4. Both solutions were each dried over MgSO₄ and evaporated to yield 2.4 g of a single stereoisomer, [α]_D²⁴=+10.68° (9.73 mg in methanol (2 mL)) and 2.5 g of the other single stereoisomer, [α]_D²⁴=-8.70° (9.88 mg in methanol (2 mL)).

10

Example 7

Compounds of formula (B)

7A. To a cold (0°C) suspension of 4-acetamidobenzenesulfonyl chloride (4.0 g, 17 mmol) in CH₂Cl₂ (40 mL) was added pyridine (1.7 mL, 20 mmol) and DMAP (209 mg, 1.7 mmol). (A clear solution resulted). Anhydrous methylamine was bubbled into the solution for 1 hour at 0°C, and then the solution was allowed to stir at 25°C for 2 hours. The solution was extracted with 1M NaOH (3x15 mL) and the combined extracts were adjusted to pH 6 at 0°C with 3M HCl. The product, which precipitated as fluffy white crystals, was filtered and washed with cold water to afford 3.2 g (82%) of 4-acetamido-N-methylbenzenesulphonamide: ¹H NMR (300 MHz, MeOH) δ 2.35 (s, 3H), 2.70 (s, 3H), 7.96 (s, 4H).

25

7B. A mixture of 4-acetamido-N-methylbenzenesulphonamide (3.2 g, 14 mmol) and 100 mL of 1M HCl was refluxed under argon for 3 hours. After cooling to 25°C, CH₂Cl₂ (10 mL) was added and the aqueous phase was neutralized with 1M NaOH at 0°C. The aqueous phase was separated and extracted with CH₂Cl₂ (2x25 mL). The combined organic phases were washed with brine (10 mL), dried (Na₂SO₄) and concentrated to afford 1.5 g (58%) of a compound of formula (B) where R⁴ is N-methylsulfonamide as a colorless solid: ¹H NMR (300 MHz, MeOH) δ 2.46 (s, 3H), 6.67-6.72 (AA' part of AA'XX' 2H), 7.48-7.52 (XX' part of AA'XX', 2H).

-69-

Example 8

Compounds of formula (C)

8A. To a cold (0°C) solution of *N*-*t*-butoxycarbonyl-L-leucine (1.4 g, 6.3 mmol) and HOBT (1.5 g, 9.8 mmol) in DMF (30 mL) was added EDCI (2.5 g, 14 mmol) in portions. After stirring for 1 hour at 0°C, the resulting solution was treated with methyl 4-aminobenzoate (1.09 mL, 6.8 mmol) and DMAP (0.32 g, 2.6 mmol). After stirring for 24 hours at 25°C, the DMF was removed in vacuo. The residue was dissolved in CH₂Cl₂ and washed with saturated NaHCO₃ solution, 1M HCl (twice), and brine. Drying over Na₂SO₄ and concentration in vacuo afforded the crude product which was purified by flash chromatography on SiO₂ (20% ethyl acetate/hexanes eluent). There was obtained 1.0 g (85%) of *N*-*t*-butoxycarbonyl-L-leucine-*N'*-(4-methoxy-carbonylphenyl)carboxamide as a foamy solid, MS (FAB) 363 (M-H)⁻.

8B. In a similar manner, the following compounds of formula (C) were prepared:

20 *N*-*t*-butoxycarbonyl-L-tryptophan-*N'*-phenylmethylcarboxamide;
N-*t*-butoxycarbonyl-L-tryptophan-*N'*-phenylcarboxamide;
25 *N*-*t*-butoxycarbonyl-L-tryptophan-*N'*-(4-methoxycarbonylphenyl)carboxamide;
N-*t*-butoxycarbonyl-L-tryptophan-*N'*-(4-ethoxycarbonylphenyl)carboxamide;
30 *N*-*t*-butoxycarbonyl-L-leucine-*N'*-(4-(N''-methylaminosulfonyl)phenyl)carboxamide;
N-*t*-butoxycarbonyl-L-alanine-*N'*-(4-methoxycarbonylphenyl)carboxamide;
35 *N*-*t*-butoxycarbonyl-L-methionine-*N'*-(4-methoxycarbonylphenyl)carboxamide;
N-*t*-butoxycarbonyl-L-leucine-*N'*-(3-ethoxycarbonylphenyl)carboxamide;
N-*t*-butoxycarbonyl-L-leucine-*N'*-(2-methoxycarbonylphenyl)carboxamide;
40 *N*-*t*-butoxycarbonyl-L-leucine-*N'*-(4-(1-methylethoxy)carbonylphenyl)carboxamide;

-70-

- N*-*t*-butoxycarbonyl-L-leucine-*N'*-
(aminosulfonyl)phenyl)carboxamide;
- N*-*t*-butoxycarbonyl-L-leucine-*N'*- (4-methoxycarbonylmethyl-
phenyl)carboxamide;
- 5 *N*-*t*-butoxycarbonyl-L-pyridin-3-ylalanine-*N'*- (4-methoxy-
carbonylphenyl)carboxamide;
- N*-*t*-butoxycarbonyl-L-cyclohexylglycine-*N'*- (4-methoxy-
carbonylphenyl)carboxamide;
- N*-*t*-butoxycarbonyl-L-isoleucine-*N'*- (4-
10 methoxycarbonylphenyl)carboxamide;
- N*-*t*-butoxycarbonyl-L-O-benzylthreonine-*N'*- (4-methoxy-
 carbonylphenyl)carboxamide;
- N*-*t*-butoxycarbonyl-L-*t*-leucine-*N'*- (4-
 methoxycarbonylphenyl)carboxamide;
- 15 *N*-*t*-butoxycarbonyl-L-leucine-*N'*- (4-cyanophenyl)carboxamide;
- N*-*t*-butoxycarbonyl-L-leucine-*N'*- (4-(*N*'-(2-dimethylaminoethyl-
 carbamoyl)carboxamide; and
- N*-*t*-butoxycarbonyl-L-leucine-*N'*- (4-(*N*'-(3-dimethylamino-
 propyl)carbamoyl)phenyl)carboxamide.
- 20
- 8C. In a similar manner, the following compounds of formula (C) are prepared:
- N*-*t*-butoxycarbonyl-L-tryptophan-*N'*- (4-nitrophenyl)carboxamide;
- N*-*t*-butoxycarbonyl-L-tryptophan-*N'*- (4-aminophenyl)carboxamide;
- 25 *N*-*t*-butoxycarbonyl-L-leucine-*N'*- (4-
 methylsulfonylphenyl)carboxamide;
- N*-*t*-butoxycarbonyl-L-leucine-*N'*- (4-
 ethylsulfonylphenyl)carboxamide; and
- N*-*t*-butoxycarbonyl-L-leucine-*N'*- (4-
30 tetrazolylphenyl)carboxamide.

Example 9

Compounds of formula (D)

- 35 9A. To a cold (0°C) solution of *N*-*t*-butoxycarbonyl-L-leucine-*N'*-phenylcarboxamide (3.4 g, 11 mmol) in dry CH₂Cl₂ (10 mL) was added TFA (2 mL). The solution was allowed to stir at 25°C

-71-

for 6 hours and was then concentrated in vacuo. The residue was partitioned between CH₂Cl₂ and H₂O and the aqueous layer was basified at 0°C with saturated K₂CO₃ solution. The organic phase was separated and the aqueous layer was extracted three times with CH₂Cl₂. The combined organic layers were washed with brine and dried over Na₂SO₄. Concentration afforded L-leucine-N'-phenylcarboxamide.

9B. In a similar manner, the following compounds are prepared:

L-leucine-N'-(4-methoxycarbonylphenyl)carboxamide;
L-tryptophan-N'-phenylmethylcarboxamide;
L-tryptophan-N'-phenylcarboxamide;
L-tryptophan-N'-(4-methoxycarbonylphenyl)carboxamide;
L-tryptophan-N'-(4-ethoxycarbonylphenyl)carboxamide;
L-leucine-N'-(4-(N''-methylaminosulfonyl)phenyl)carboxamide;
L-alanine-N'-(4-methoxycarbonylphenyl)carboxamide;
L-methionine-N'-(4-methoxycarbonylphenyl)carboxamide;
L-leucine-N'-(3-ethoxycarbonylphenyl)carboxamide;
L-leucine-N'-(2-methoxycarbonylphenyl)carboxamide;
L-leucine-N'-(4-(1-methylethoxy)carbonylphenyl)carboxamide;
L-leucine-N'-(aminosulfonyl)phenyl)carboxamide;
L-leucine-N'-(4-methoxycarbonylmethylphenyl)carboxamide;
L-pyridin-3-ylalanine-N'-(4-methoxycarbonylphenyl)carboxamide;
L-spirocyclopentylglycine-N'-(4-methoxycarbonylphenyl)-
carboxamide;
L-cyclohexylglycine-N'-(4-methoxycarbonylphenyl)carboxamide;
L-isoleucine-N'-(4-methoxycarbonylphenyl)carboxamide;
L-O-benzylthreonine-N'-(4-methoxycarbonylphenyl)carboxamide;
L-t-leucine-N'-(4-methoxycarbonylphenyl)carboxamide;
L-leucine-N'-(4-cyanophenyl)carboxamide;
L-leucine-N'-(4-(N''-(2-dimethylaminoethyl)carbamoyl)phenyl)-
carboxamide; and
L-leucine-N'-(4-(N''-(3-dimethylaminopropyl)carbamoyl)phenyl)-
carboxamide.

-72-

9C. In a similar manner, the following compounds of formula (D) are prepared:

L-tryptophan-N'-(4-nitrophenyl)carboxamide;
L-tryptophan-N'-(4-aminophenyl)carboxamide;
5 L-leucine-N'-(4-methylsulfonylphenyl)carboxamide;
L-leucine-N'-(4-ethylsulfonylphenyl)carboxamide; and
L-leucine-N'-(4-tetrazolylphenyl)carboxamide.

Example 10

10 Compounds of formula (Ia)

10A. To a cold (0°C) solution of 2-(hydroxy)(quinolin-2-ylthiomethyl)-phosphinoylmethyl-4-methylpentanoic acid (0.20 g, 0.54 mmol) in THF (6 mL) was added 1,1'-carbonyldiimidazole (0.12 g, 0.7 mmol). The mixture was stirred for 75 minutes at 15 0°C and was then treated with L-tryptophan-N'-(4-ethoxy-carbonylphenyl)carboxamide (0.22 g, 0.62 mmol) and stirred at 25°C for 15 hours. The THF was evaporated and the residue was dissolved in ethyl acetate (60 mL). The solution was washed with H₂O (10 mL), brine (10 mL), and dried over MgSO₄.
20 Concentration was followed by reverse phase HPLC using a gradient of acetonitrile and 50 mM NH₄OAc buffer afforded 30 mg of N-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoyl)-L-tryptophan-N'-(4-ethoxycarbonylphenyl)-carboxamide as an off white solid, MS(FAB) 701 (M-H)⁺ (mixture 25 of diastereomers).

10B. In a similar manner, the following compounds of formula (Ia) were prepared:

30 N-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoyl)-L-tryptophan-N'-(4-methoxy-carbonylphenyl)carboxamide,
MS(FAB) 687 (M+H)⁺;
N-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoyl)-L-alanine-N'-(4-methoxy-carbonylphenyl)carboxamide,
35 MS(FAB) 572 (M+H)⁺;
N-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-

-73-

4-methylpentanoyl)-L-methionine-N'-(4-methoxy-carbonylphenyl)carboxamide,

MS (FAB) 632 (M+H)⁺;

5 N- (2- (hydroxy) (quinolin-2-ylthiomethyl) phosphinoylmethyl-4-methylpentanoyl)-L-leucine-N'-(4-methoxy-carbonylphenyl)carboxamide;

MS (FAB) 614 (M+H)⁺;

10 N- (2- (hydroxy) (quinolin-2-ylthiomethyl) phosphinoylmethyl-4-methylpentanoyl)-L-leucine-N'-(3-ethoxy-carbonylphenyl)carboxamide,

¹H NMR (300 MHz, MeOH) δ 0.73-1.01 (m, 12H), 1.28-2.00 (m, 14H), 2.4-3.61 (m, 2H), 4.27-4.45 (m, 3H), 7.23-7.44 (m, 3H), 7.65-7.98 (m, 6H), 8.29 (s, 0.5H), 8.50 (s, 0.5H);

15 N- (2- (hydroxy) (quinolin-2-ylthiomethyl) phosphinoylmethyl-4-methylpentanoyl)-L-leucine-N'-(2-methoxy-carbonylphenyl)carboxamide,

¹H NMR (300 MHz, MeOH) δ 0.78-0.99 (m, 13H), 1.3-2.4 (m, 7H), 2.90-3.05 (m, 1H), 3.5-3.75 (m, 2H), 3.89, 3.90, 3.94 (3s, 3H total), 4.35-3.50 (m, 1H), 7.05-8.10 (m, 11H), 8.32, 8.55, 8.60 (3d, J=8.7, 1H);

20 N- (2- (hydroxy) (quinolin-2-ylthiomethyl) phosphinoylmethyl-4-methylpentanoyl)-L-leucine-N'-(4-(1,1-dimethyl-ethoxycarbonylphenyl)carboxamide,

MS (FAB) 642 (MH)⁺;

25 N- (2- (hydroxy) (quinolin-2-ylthiomethyl) phosphinoylmethyl-4-methylpentanoyl)-L-leucine-N'-(4-aminosulfonyl-phenyl)carboxamide,

¹H NMR (300 MHz, MeOH) δ 0.76 (d, J=6.5, 3H), 0.81 (d, J=6.5, 3H), 0.85-1.1 (m, 7H), 1.2-2.1 (m, 7H), 2.92-2.95 (m, 1H), 3.45-3.70 (m, 2H), 4.35-4.45 (m, 1H), 7.28 (d, J=8.7, 1H), 7.45 (t, J=8.7, 1H), 7.68 (t, J=8.7, 1H), 7.7-7.8 (m, 3H), 7.87 (d, J=8.7, 1H), 7.95-8.1 (m, 3H);

30 N- (2- (hydroxy) (quinolin-2-ylthiomethyl) phosphinoylmethyl-4-methylpentanoyl)-L-leucine-N'-(4-methoxycarbonylmethyl-phenyl)carboxamide,

MS (FAB) 628 (MH)⁺.

- 74 -

10C. In a similar manner, the following compounds of formula (Ia) are prepared:

- 5 *N*-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-
 5-phenylpentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 10 *N*-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-
 4-phenylbutanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 15 *N*-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-
 3-phenylpropanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 20 *N*-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl)-
 pentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 25 *N*-(2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
 5-phenylpentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 30 *N*-(2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
 4-phenylbutanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 35 *N*-(2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
 3-phenylpropanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 40 *N*-(2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
 3-cyclohexylpropanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 45 *N*-(2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl)-
 pentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 50 *N*-(2-(hydroxy)(naphth-1-ylthiomethyl)phosphinoylmethyl-
 5-phenylpentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 55 *N*-(2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
 5-phenylpentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;
- 60 *N*-(2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-
 4-phenylbutanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl)carboxamide;

- 75 -

- phenyl)carboxamide;
- 5 *N*-(2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-3-phenylpropanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-phenyl)carboxamide;
- 10 *N*-(2-(hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl-3-cyclohexylpropanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-phenyl)carboxamide;
- 15 *N*-(2-((hydroxy)(naphth-2-ylthiomethyl)phosphinoylmethyl)-pentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-phenyl)carboxamide;
- 20 *N*-(2-(hydroxy)(phenylthiomethyl)phosphinoylmethyl-4-phenylbutanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-phenyl)carboxamide;
- 25 *N*-(2-(hydroxy)(phenylthiomethyl)phosphinoylmethyl-3-phenylpropanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-phenyl)carboxamide; and
- 30 *N*-(2-((hydroxy)(phenylthiomethyl)phosphinoylmethyl)-pentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-phenyl)carboxamide.

35 10D. A solution of *N*-(2-(hydroxy)(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoyl)-L-tryptophan-*N'*-(4-methoxycarbonylphenyl)carboxamide in THF (2 mL) and 1M NaOH (1 mL) was stirred for 24 hours at 25°C. The organic solvents were evaporated, and the residue dissolved in ethyl acetate/H₂O. The aqueous phase was acidified with 1M HCl and the separated aqueous phase was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried (MgSO₄) and concentrated to 27 mg of *N*-(2-(hydroxy)-(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoyl)-L-tryptophan-*N'*-(4-carboxyphenyl)carboxamide as a

-76-

yellow powder.

10E. In a similar manner, but starting with *N*-(2-(hydroxy)-
(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoyl)-
L-leucine-*N'*-(4-methoxy-carbonylphenyl)carboxamide (30 mg,
0.048 mmol) there was obtained 10 mg of *N*-(2-(hydroxy)-
(quinolin-2-ylthiomethyl)phosphinoylmethyl-4-methylpentanoyl)-
L-leucine-*N'*-(4-carboxyphenyl)carboxamide as a semisolid after
trituration with ethyl acetate;

10 ^1H NMR¹ (300 MHZ, MeOH) 0.81-1.02 (m, 12H), 1.1-2.3 (m, 10H),
2.82-3.00 (m, 1H), 3.49, 3.56 (2s, 2H), 3.5-3.8 (m, 2H), 4.45-
4.55 (m, 1H), 7.09 (d, J=8.2, 1H), 7.19 (d, J=8.2, 1H), 7.45 (t,
J=8.2, 1H) 7.45-7.6 (m, 3H), 7.65-7.80 (m, 1H), 7.82-7.98 (m,
2H), 8.10-8.20 (m, 1H).

15

Example 11

Compounds of formula (Fa)

11A. To 4-methylpentanoic acid (25 g, 0.215 mmol) in a 25°C
water bath, thionyl chloride (20.4 mL, 1.3 g) was slowly
added. Then the mixture was heated at 50°C under argon for 3
hours (until the evolution of gas had stopped). The crude
reaction mixture was distilled at atmospheric pressure to give
4-methylpentanoyl chloride (25.3 g, 87.3%), b.p. 143°C.

25

11B. In a similar manner, but replacing 4-methylpentanoic acid
with 5-phenylpentanoic acid (5 g), 5-phenylpentanoyl chloride
was prepared (4.4 g), as a colorless liquid, b.p. 91°-93°C.

30

Example 12

Compounds of formula (Fb)

12A. To a suspension of 60% NaH (836 mg, 1.5 eq.) in toluene
(200 mL) at room temperature under argon was added L-(+)-2,10-
camphor sultam (3.0 g, 13.9 mmol) portion-wise. The mixture
35 was stirred vigorously at room temperature for one hour. Then
4-methylpentanoyl chloride was carefully added dropwise to the
solution at 0°C. After stirring the reaction at room

- 77 -

temperature for 3 hours, the reaction was quenched with 10 mL of water, and 70 mL of ether was added. The reaction mixture was first washed with 0.5N HCl (2x50 mL), then 5% K₂CO₃ (3x50 mL) and finally with brine (1x50 mL). The organic layer was dried over MgSO₄, filtered and evaporated to dryness.

Purification by column chromatography (1:6 ethyl acetate/petroleum ether as eluant) gave *N*-4-methylpentanoyl-L-(+)-2,10-camphor sultam (3.39 g, 78%).

- 10 12B. In a similar manner, but replacing 4-methylpentanoyl chloride with the appropriate chloride, the following compounds of formula (Fb) were prepared: ---
- N-3-phenylpropanoyl-L-(+)-2,10-camphor sultam, MS: 347 (M⁺);
- N-5-phenylpentanoyl-L-(+)-2,10-camphor sultam, MS: 375 M⁺;
- 15 N-pentanoyl-L-(+)-2,10-camphor sultam, MS: 300 (M+H)⁺.

Example 13

Compounds of formula (Fc)

- 13A. To a solution of *N*-4-methylpentanoyl-L-(+)-2,10-camphor sultam (3.39 g, 10.8 mmol) in 75 mL of dry THF at -78°C under argon was added NaN(TMS)₂ (1.0 M in THF, 11.34 mL, 1.05 eq.) dropwise over five minutes. After stirring at -78°C for 1 hour, hexamethylphosphoramide (5 mL) was added to the mixture, followed by t-butylbromoacetate (5.2 mL, 3 eq), then 400 mg of tetra *n*-butylammonium iodide was added in one portion. The resulting solution was kept at -78°C under argon overnight. The next morning, the reaction was quenched with water (100 mL), and then it was extracted with ether (3x100 mL). The combined ether layers were washed with brine, then dried over Na₂SO₄, filtered and concentrated. Purification by column chromatography (5:95 ethyl acetate/petroleum ether to 10:90 ethyl acetate/petroleum ether as eluant) gave *N*-(4-methyl-2-t-butoxycarbonylmethyl)pentanoyl-L-(+)-2,10-camphor sultam (4 g, 86.5%).

- 35 13B. In a similar manner, but replacing *N*-4-methylpentanoyl-L-(+)-2,10-camphor sultam with the appropriate compound of ...

-78-

formula (Fb), the following compounds of formula (Fc) were prepared:

N-(3-phenyl-2-*t*-butoxycarbonylmethyl)propanoyl-L-(-)-2,10-camphor sultam, MS: 461 (M^+);

5 *N*-(5-phenyl-2-*t*-butoxycarbonylmethyl)pentanoyl-L-(-)-2,10-camphor sultam, MS: 490.1 ($M+H$)⁺;

N-(2-*t*-butoxycarbonylmethyl)pentanoyl-L-(-)-2,10-camphor sultam, MS: 414 ($M+H$)⁺.

10

Example 14

Compounds of formula (F)

14A. To a stirred solution of *N*-(4-methyl-2-*t*-butoxycarbonylmethyl)pentanoyl-L-(-)-2,10-camphor sultam (5.45 g, 12.7 mmol) in 50% aqueous THF (150 mL) at 0°C under argon was added LiOH·H₂O crystals (2.14 g, 4 eq.) followed by 30% H₂O₂ (11.5 mL). Then the ice-bath was removed and the resulting emulsion was stirred for 3 hours before it had turned clear. Most of the THF was removed under reduced pressure at 35°C. Then CH₂Cl₂ (150 mL) was added and with stirring 4N HCl was added to pH=2. After adding NaCl, the aqueous layer was further extracted with CH₂Cl₂ (3x150 mL). The CH₂Cl₂ was removed under reduced pressure at 35°C and then the residue was taken up in ethyl acetate (150 mL). This solution was then extracted with 5% K₂CO₃ (3x50 mL) and the combined extracts were washed with ether (50 mL). Then CH₂Cl₂ was added to the aqueous layer and with stirring with NaCl, the aqueous layer was extracted with CH₂Cl₂ (3x70 mL) and the combined extracts were dried over Na₂SO₄, filtered and concentrated to give (2*R*)-4-methyl-2-*t*-butoxycarbonylmethyl-pentanoic acid as a colorless oil (2.95 g, quantitative yield).

14B. In a similar manner, but replacing *N*-(4-methyl-2-*t*-butoxycarbonylmethyl)pentanoyl-L-(-)-2,10-camphor sultam with the appropriate compound of formula (Fc), the following compounds of formula (F) were prepared:

35 (2*R*)-3-phenyl-2-*t*-butoxycarbonylmethyl-propanoic acid,

MS: 265 ($M+H$)⁺;

-79-

(2R)-5-phenyl-2-t-butoxycarbonylmethyl-pentanoic acid,

MS: 293.1 (M+H)⁺;

(2R)-2-t-butoxycarbonylmethyl-pentanoic acid,

(colorless oil, 1.09 g).

5

14C. (2R)-3-Phenyl-2-t-butoxycarbonylmethyl-propanoic acid (55 mg) was taken up in glacial acetic acid (20 mL) and PtO₂ (25 mg) was added in acetic acid. Then the beaker was placed in a Parr bomb, it was evacuated and charged with 100 psi of H₂.
10 After stirring for 3 days, the mixture was suction filtered through a 1 cm bed of Celite. The filtrate was then concentrated to a yellow oil, -(2R)-3-cyclohexyl-2-t-butoxycarbonylmethyl-propanoic acid (56 mg),
MS: 269.5 (M-H)⁺.

15

Example 15

Compounds of formula (Ib)

15A. To a solution of 4-methyl-2-t-butoxycarbonylmethyl-pentanoic acid (0.28 g, 1.2 mmol) in DMF (5 mL) containing HOBT (0.22 g, 1.8 mmol) was added EDCI (0.31 g, 1.8 mmol).
20 The mixture was stirred at 0°C for 1 hour and was then treated with L-cyclohexylglycine-N'-(4-methoxycarbonylphenyl)-carboxamide (1.2 mmol) and DMAP (27 mg, 0.24 mmol). Stirring was continued for 24 hours at 25°C and then the DMF was
25 evaporated. The residue was dissolved in CH₂Cl₂ (20 mL) and the solution was washed with 1M HCl (10 mL), saturated NaHCO₃ (10 mL), brine (10 mL) and was dried over Na₂SO₄. Concentration in vacuo afforded an oil which was purified by flash chromatography on SiO₂ using 20% ethyl acetate/hexanes as element.
30 There was obtained 0.22 g (22%) of N-(4-methyl-2-t-butoxycarbonylmethyl-pentanoyl)-L-cyclohexylglycine-N'-(4-methoxycarbonylphenyl)carboxamide as a solid, MS(FAB) 503 (MH)⁺.

30

35 15B. In a similar manner, the following compounds were prepared:

N-(4-methyl-2-t-butoxycarbonylmethylpentanoyl)-L-pyridin-

-80-

- 3-ylalanine-*N'*-(4-methoxycarbonylphenyl)carboxamide;
N-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-*O*-benzyl-
threonine-*N'*-(4-methoxycarbonylphenyl)carboxamide;
5 *N*-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-isoleucine-
 N'-(4-methoxycarbonylphenyl)carboxamide;
 N-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-leucine-
 N'-(4-methoxycarbonylphenyl)carboxamide;
 N-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-*t*-leucine-
 N'-(4-methoxycarbonylphenyl)carboxamide;
10 *N*-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-leucine-
 N'-(4-cyanophenyl)carboxamide;
 N-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-leucine-
 N'-(4-(*N'*-(3-dimethylaminopropyl)carbamoyl)phenyl)-
 carboxamide;
15 *N*-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-leucine-
 N'-(4-(*N''*-(2-dimethylaminoethyl)carbamoyl)phenyl)-
 carboxamide;
 N-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-leucine-
 N'-(4-aminosulfonylphenyl)carboxamide;
20 *N*-(4-methyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-leucine-
 N'-(4-methylaminosulfonylphenyl)carboxamide;
 N-(2-*t*-butoxycarbonylmethylpentanoyl)-L-leucine-
 N'-(4-methoxycarbonylphenyl)carboxamide;
 N-(3-phenyl-2-*t*-butoxycarbonylmethylpropanoyl)-L-leucine-
25 *N'*-(4-methoxycarbonylphenyl)carboxamide;
 N-(3-cyclohexyl-2-*t*-butoxycarbonylmethylpropanoyl)-L-leucine-
 N'-(4-methoxycarbonylphenyl)carboxamide;
 N-(4-phenyl-2-*t*-butoxycarbonylmethylbutanoyl)-L-leucine-
 N'-(4-methoxycarbonylphenyl)carboxamide; and
30 *N*-(5-phenyl-2-*t*-butoxycarbonylmethylpentanoyl)-L-leucine-
 N'-(4-methoxycarbonylphenyl)carboxamide.

-81-

Example 16

Compounds of formula (Ic)

16A. To a cold (0°C) solution of *N*-(4-methyl-2-*t*-butoxy-carbonylmethyl-pentanoyl)-L-cyclohexylglycine-*N'*-(4-methoxycarbonylphenyl)carboxamide (70 mg, 0.14 mmol) in CH_2Cl_2 , (2 mL) was added TFA (0.5 mL). After stirring for 5 hours at 25°C , the solution was concentrated in vacuo and the product was purified by reverse phase HPLC using a gradient of acetonitrile and 50 mM NH_4OAc buffer to provide 44 mg (71%) of *N*-(4-methyl-2-carboxymethylpentanoyl)-L-cyclohexylglycine-*N'*-(4-methoxycarbonylphenyl)carboxamide as a white solid, MS(FAB) 445. ($\text{M}-\text{H}$) $^{+}$.

16B. In a similar manner, the following compounds were prepared:

N-(4-methyl-2-carboxymethylpentanoyl)-L-isoleucine-*N'*-(4-methoxycarbonylphenyl)carboxamide,
MS(FAB) 419 ($\text{M}-\text{H}$) $^{+}$;

N-(4-methyl-2-carboxymethylpentanoyl)-L-leucine-*N'*-(4-methoxy-carbonylphenyl)carboxamide, MS(FAB) 419 ($\text{M}-\text{H}$) $^{+}$;

N-(4-methyl-2-carboxymethylpentanoyl)-L-*t*-leucine-*N'*-(4-methoxycarbonylphenyl)carboxamide;

N-(4-methyl-2-carboxymethylpentanoyl)-L-leucine-*N'*-(4-cyano-phenyl)carboxamide,

^1H NMR (300 MHz, MeOH) δ 0.84-0.99 (m, 12H), 1.15-1.82 (m, 6H), 2.36-2.41 (m, 1H), 2.52-2.65 (m, 1H), 2.8-2.95 (m, 1H), 4.49-4.54 (m, 1H), 7.4-7.9 (m, 4H);

N-(4-methyl-2-carboxymethylpentanoyl)-L-leucine-*N'*-(4-amino-sulfonylphenyl)carboxamide,

^1H NMR (300 MHZ, MeOH) δ 0.85-1.00 (m, 12H), 1.1-1.3 (m, 2H), 1.52-1.85 (m, 4H), 2.31-2.95 (m, 3H), 4.49-4.55 (m, 1H), 7.75-7.91 (m, 4H);

N-(4-methyl-2-carboxymethylpentanoyl)-L-leucine-*N'*-(4-methyl-aminosulfonylphenyl)carboxamide, MS(FAB) 459 ($\text{M}-\text{H}$) $^{+}$;

N-(2-carboxymethylpentanoyl)-L-leucine-*N'*-(4-methoxy-carbonylphenyl)carboxamide, MS(FAB) 405 ($\text{M}-\text{H}$) $^{+}$;

N-(3-phenyl-2-carboxymethylpropanoyl)-L-leucine-*N'*-(4-methoxy-

-82-

- carbonylphenyl)carboxamide, MS(FAB) 455 (M+H)⁺;
N- (3-cyclohexyl-2-carboxymethylpropanoyl)-L-leucine-
N'- (4-methoxycarbonylphenyl)carboxamide,
 MS(FAB) 459 (M-H)⁻;
- 5 *N*- (4-phenyl-2-carboxymethylbutanoyl)-L-leucine-*N'*- (4-methoxy-
 carbonylphenyl)carboxamide, MS(FAB) 467 (M-H)⁻;
N- (4-phenyl-2-carboxymethylbutanoyl)-L-cyclohexylglycine-
N'- (4-methoxycarbonylphenyl)carboxamide;
- 10 *N*- (4-phenyl-2-carboxymethylbutanoyl)-L-t-leucine-
N'- (4-methoxycarbonylphenyl)carboxamide;
- 15 *N*- (5-phenyl-2-carboxymethylpentanoyl)-L-leucine-*N'*- (4-methoxy-
 carbonylphenyl)carboxamide, MS(FAB) 481 (M-H)⁻; and
N- (4-methyl-2-carboxymethylpentanoyl)-L-O-benzylthreonine-
N'- (4-methoxycarbonylphenyl)carboxamide,
 MS(FAB) 497 (M-H)⁻.

- 16C. In a similar manner, but triturating the crude product with ether and then decanting the ether to yield the following compounds as TFA salts:
- 20 *N*- (4-methyl-2-carboxymethylpentanoyl)-L-pyridin-3-ylalanine-
N'- (4-methoxycarbonylphenyl)carboxamide,
 MS(FAB) 456 (M+H)⁺;
- N- (4-methyl-2-carboxymethylpentanoyl)-L-leucine-
N'- (4-(*N*'-(3-dimethylaminopropyl)carbamoyl)phenyl)-
 carboxamide, MS(FAB) 491 (M+H)⁺; and
- 25 *N*- (4-methyl-2-carboxymethylpentanoyl)-L-leucine-
N'- (4-(*N*'-(2-dimethylaminoethyl)carbamoyl)phenyl)-
 carboxamide, MS(FAB) 491 (M+H)⁺.

- 30 16D. A mixture of *N*- (4-methyl-2-t-butoxycarbonylmethyl-
 pentanoyl)-L-O-benzylthreonine-*N'*- (4-methoxycarbonylphenyl)-
 carboxamide (60 mg) and Pd/C in ethyl acetate/THF (1:1, 25 mL)
 was hydrogenated overnight at 1 atm pressure. Filtration
 through Celite, concentration of the filtrate, and trituration
 of the residue with ether/hexanes produced *N*- (4-methyl-2-t-
 butoxycarbonylmethyl-pentanoyl)-L-threonine-*N'*- (4-
 methoxycarbonyl-phenyl)carboxamide, MS(FAB) 407 (M-H)⁻.

- 83 -

16E. By following the procedure of part A and substituting *N*-(4-methyl-2-t-butoxycarbonylmethyl-pentanoyl)-L-cyclohexylglycine-*N'*-(4-methoxycarbonylphenyl)carboxamide with the following:

- 5 *N*-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-lysine-*N'*-(4-(ethoxycarbonyl)phenyl)carboxamide;
- 10 *N*-(2R-(t-butoxycarbonyl)methyl-5-(phenyl)pentanoyl)-L-lysine-*N'*-(4-(ethoxycarbonyl)phenyl)carboxamide;
- 15 *N*-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-(N_ε-isopropyl)lysine-*N'*-(4-(ethoxycarbonyl)phenyl)carboxamide;
- 20 *N*-(2R-(t-butoxycarbonyl)methyl-4-(phenyl)butanoyl)-L-cyclohexylglycine-*N'*-(4-(*N'',N''*-dimethylaminoethylaminosulfonyl)phenyl)carboxamide;
- 25 *N*-(2R-(t-butoxycarbonyl)methyl-5-(phenyl)pentanoyl)-L-(*N,N'*-diethylguanido)lysine-*N'*-(4-(ethoxycarbonyl)phenyl)carboxamide;
- 30 *N*-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-*N'*-(4-(methylthio)phenyl)carboxamide;
- 35 *N*-(2R-(t-butoxycarbonyl)methyl-5-biphen-4-yl)pentanoyl)-L-S-((4-cyanophenyl)methyl)penicillamine-*N'*-(phenyl)carboxamide;
- 40 *N*-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-cyclohexylglycine-*N'*-(2-(4-aminosulfonyl)phenylethyl)carboxamide;
- 45 *N*-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-cyclohexylglycine-*N'*-(3-(morpholin-4-yl)propyl)carboxamide;
- 50 *N*-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-*N'*-(4-(methylaminosulfonyl)phenyl)carboxamide;
- 55 *N*-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-cyclohexylglycine-*N'*-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)carboxamide;
- 60 *N*-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-cyclohexylglycine-*N'*-(4-(*N'',N''*-

- 84 -

dimethylaminoethylaminosulfonyl) phenyl) carboxamide; and
 N- (2R- (t-butoxycarbonyl)methyl-5- (biphen-4-yl) pentanoyl)-L-t-
 leucine-N'- (4- ((3- (morpholin-4-yl) propyl) aminosulfonyl)-
 phenyl) carboxamide,

5 there are obtained:

N- (2R,S) - (N"-formyl-N"-hydroxyamino)methyl-4-
 (methyl) pentanoyl) -

L-leucine-N'- (4- (methoxycarbonyl) phenyl) carboxamide: MS:
 434.2 (M-H)⁺ 388 (M-HCO-OH);

10 N- (2R- (N"-hydroxycarbamoyl)methyl-4- (methyl) pentanoyl)
 D,L-norvaline-N'- (4- ((2- (dimethylamino) ethyl)-
 carbamoyl) phenyl) carboxamide: MS: 478 (M+H)⁺;

N- (2R-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
 L-lysine-N'- (4- (ethoxycarbonyl) phenyl) carboxamide:
 15 MS: 588.3 (M+H)⁺;

N- (2R-carboxymethyl-5- (phenyl) pentanoyl) -
 L-lysine-N'- (4- (ethoxycarbonyl) phenyl) carboxamide:
 MS: 512.3 (M+H)⁺;

20 N- (2R-carboxymethyl-5- (biphen-4-yl) pentanoyl) -
 L- (N_ε-isopropyl) lysine-N'- (4- (ethoxycarbonyl) phenyl)-
 carboxamide: MS: 630 (M+H)⁺;

N- (2R-carboxymethyl)-4- (phenyl) butanoyl) -
 L-cyclohexylglycine-N'- (4- (N^N,N^N-dimethylaminoethyl-
 aminosulfonyl) phenyl) carboxamide: MS: 586 (M+H)⁺;

25 N- (2R-carboxymethyl-5- (phenyl) pentanoyl) -
 L- (N,N'-diethylguanido) lysine-N'- (4- (ethoxycarbonyl)-
 phenyl) carboxamide: MS: 610.4 (M+H)⁺;
 N- (2R-carboxymethyl)-5- (biphen-4-yl) pentanoyl)-L-t-leucine-N'-
 (4- (methylthio) phenyl) carboxamide: FAB-MS (M+Na)⁺ calc.
 30 569.2450; found: 569.2461;

N- (2R-carboxymethyl-5-biphen-4-yl) pentanoyl)-L-t-leucine-N'-
 (3- (2-hydroxyethyl) phenyl) carboxamide: FAB-MS (M+H)⁺
 calc. 545.3015; found: 545.3021;

35 N- (2R-carboxymethyl-5-biphen-4-yl) pentanoyl)L-S- ((4-
 cyanophenyl) methyl) penicillamine-N'- (phenyl) carboxamide:
 FAB-MS (M+H)⁺ calc. 634.2740; found: 634.2749;

N- (2R-carboxymethyl-5- (biphen-4-yl) pentanoyl)-L-

-85-

cyclohexylglycine-*N'*-(2-(4-aminosulfonyl)phenylethyl)-carboxamide: FAB-MS (M+H)⁺ calc. 634.2951; found: 634.2963;

5 *N*-(2*R*-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-cyclohexylglycine-*N'*-(3-(morpholin-4-yl)propyl)-carboxamide: FAB-MS (M+H)⁺ calc. 578.3594; found: 578.3583;

10 *N*-(2*R*-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-*N'*-(4-(methylaminosulfonyl)phenyl)carboxamide: ¹H NMR (300 MHz, acetone-d₆) δ 9.73 (br, s, 1H), 7.91 (d, 2H, J= 9 Hz), 7.79 (d, 2H, J= 9 Hz), 7.56 (d, 2H, J= 8 Hz), 7.25-7.45 (m, 6H), 7.19 (d, 2H, J= 8 Hz), 4.48 (d, 1H, J= 9 Hz), 2.38-3.00 (m, 9H), 1.43-1.72 (m, 4H), 1.04 (s, 9H);

15 *N*-(2*R*-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-cyclohexylglycine-*N'*-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)-carboxamide: FAB-MS (M+Cs)⁺ calc. 782.1876; found: 782.1896;

20 *N*-(2*R*-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-cyclohexylglycine-*N'*-(4-((2-dimethylamino)ethyl)aminosulfonyl)phenyl)carboxamide: FAB-MS (M+ Cs)⁺ calc. 809.2349; found: 809.2369; and

25 *N*-(2*R*-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-*N'*-(4-((3-(morpholin-4-yl)propyl)aminosulfonyl)phenyl)-carboxamide: FAB-MS (M+H)⁺ calc. 707.3478; found: 707.3489.

Example 17

Compounds of formula (Id)

17A. A solution of *N*-(4-methyl-2-carboxymethylpentanoyl)-L-leucine-*N'*-(4-methoxycarbonylphenyl)carboxamide (0.28 g, 0.66 mmol) and HOBT (0.12 g) in dry DMF (20 mL) was cooled to 0°C and treated with EDCI (0.32 g). After stirring 0.5 hours at 0°C, O-benzylhydroxylamine (0.30 mL) was added and the reaction was allowed to warm to 25°C overnight. The DMF was removed in vacuo and the residue was taken up in CH₂Cl₂ and washed with 5% HCl/5% NaHCO₃, and brine and the solution was dried over Na₂SO₄. After concentration, the product was

-86-

purified by flash chromatography (SiO_2 , $R_f=0.6$, 10% MeOH/CH₂Cl₂). The product containing fractions were further purified by trituration with CH₂Cl₂ to give N-(4-methyl-2-(N''-benzyloxycarbamoyl)methylpentanoyl)-L-leucine-N'-(4-methoxycarbonylphenyl)carboxamide as a solid, mp 198-199°C.

5 17B. In a similar manner, the following compounds were prepared:

10 N-(2-(N''-benzyloxycarbamoyl)methylpentanoyl)-L-leucine-N'-(4-methoxycarbonylphenyl)carboxamide;
N-(4-phenyl-2-(N''-benzyloxycarbamoyl)methylbutanoyl)-L-leucine-N'-(4-methoxycarbonylphenyl)carboxamide; and
N-(4-methyl-2-(N''-benzyloxycarbamoyl)methylpentanoyl)-L-tryptophan-N'-(4-methoxycarbonylphenyl)carboxamide.

15 17C. N-(4-Methyl-2-(N''-benzyloxycarbamoyl)methylpentanoyl)-L-leucine-N'-(4-methoxycarbonylphenyl)carboxamide (210 mg) was hydrolyzed with 1M NaOH (1.4 mL) at 50-60°C for 2 hour in THF (20 mL) and MeOH (5 mL). The organic solvents were evaporated and the residue was taken up in 10 mL H₂O and washed with ether (2x10 mL). The aqueous phase was acidified to pH 2 with 10% HCl and extracted with ethyl acetate (3x10 mL). The combined extracts were washed with brine dried (Na₂SO₄), and concentrated to afford N-(4-methyl-2-(N''-benzyloxycarbamoyl)-methylpentanoyl)-L-leucine-N'-(4-carboxyphenyl)carboxamide (110 mg).

Example 18

Compounds of formula (Ie)

30 18A. To a solution of N-(4-phenyl-2-(N''-benzyloxy-carbamoyl)methyl-butanoyle)-L-leucine-N'-(4-methoxycarbonyl-phenyl)carboxamide (25 mg) in 20 mL MeOH and 10 mL THF was added 10% Pd/C (20 mg). The suspension was hydrogenated for 1 hour and then suction filtered through Celite. Concentration afforded the product which was purified on silica (2.5% MeOH/CH₂Cl₂) to give 8 mg of N-(4-phenyl-2-(N''-hydroxy-carbamoyl)methyl-butanoyle)-L-leucine-N'-(4-methoxycarbonyl)-

-87-

phenyl)carboxamide, MS (FAB) 482 (M-H)⁻.

18B. In a similar manner, the following compounds were prepared:

- 5 *N*-(4-methyl-2-(*N*''-hydroxycarbamoyl)methylpentanoyl)-
 L-leucine-*N'*-(4-carboxyphenyl) carboxamide;
 N-(4-methyl-2-(*N*''-hydroxycarbamoyl)methylpentanoyl)-
 L-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide,
 MS (FAB) 436 (M+H)⁺;
- 10 *N*-(2-(*N*''-hydroxycarbamoyl)methylpentanoyl)-
 L-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide,
 MS (FAB) 420 (M-H)⁻;
- N*-(4-phenyl-2-(*N*''-hydroxycarbamoyl)methylbutanoyl)-
 L-t-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide;
- 15 *N*-(4-phenyl-2-(*N*''-hydroxycarbamoyl)methylbutanoyl)-
 L-cyclohexylglycine-*N'*-(4-methoxycarbonyl-
 phenyl) carboxamide;
 N-(4-phenyl-2-(*N*''-hydroxycarbamoyl)methylbutanoyl)-
 L-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide;
- 20 *N*-(4-methyl-2-(*N*''-hydroxycarbamoyl)methylpentanoyl)-
 L-t-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide;
 N-(4-methyl-2-(*N*''-hydroxycarbamoyl)methylpentanoyl)-
 L-tryptophan-*N'*-(4-methoxycarbonylphenyl) carboxamide,
 MS (FAB) 507 (M-H)⁻; and
- 25 *N*-(4-phenyl-2-(*N*''-hydroxycarbamoyl)methylbutanoyl)-
 L-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide.

18C. In a similar manner, the following compounds are prepared:

- 30 *N*-(3-phenyl-2-(*N*''-hydroxycarbamoyl)methylpropanoyl)-
 L-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide;
- N*-(5-phenyl-2-(*N*''-hydroxycarbamoyl)methylpentanoyl)-
 L-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide; and
- N*-(3-cyclohexyl-2-(*N*''-hydroxycarbamoyl)methylpropanoyl)-
 L-leucine-*N'*-(4-methoxycarbonylphenyl) carboxamide.

-88-

Example 19

Compounds of formula (Gb)

19A. To a cold (0°C) solution of diethyl isobutylmalonate (21.6 g, 0.1 mol) in 150 mL of ethanol was added a solution of KOH (5.89 g, 0.1 mol) slowly over 30 minutes. The clear 5 solution was stirred at 25°C for 60 hours. The ethanol was removed under reduced pressure and the solid residue was dissolved in 50 mL of H_2O . The aqueous solution was acidified to pH 2 with 4M HCl and extracted with ether (2x50 mL). The 10 combined extracts were dried over MgSO_4 and evaporated to provide 19.0 g (100%) of ethyl isobutylmalonate as a colorless oil.

19B. In a similar manner, the following compounds of formula 15 (Gb) are prepared: ethyl tert-butylmalonate; ethyl propylmalonate; ethyl benzylmalonate; and ethyl cyclohexymethylmalonate.

Example 20

Compounds of formulae (Gc) and (Gd)

20A. To neat ethyl isobutylmalonate (25 g, 0.13 mol) at 0°C was slowly added ice cold diethylamine (15.1 mL, 0.15 mol). After stirring for 15 minutes, formalin (11.1 mL of 37% aqueous formaldehyde) was added dropwise and the mixture was 25 allowed to stir at 25°C for 3 days. The reaction was treated with a solution of 20 g of K_2CO_3 in 40 mL of H_2O and extracted with ether (2 x 100 mL). The combined ether layers were washed with brine, dried over MgSO_4 , and evaporated at 20°C on a rotary evaporator. The crude product ethyl 4-methyl-2- 30 methylenepentanoate (containing some ether) was dissolved in 250 mL of absolute ethanol and treated with acetonitrile (250 mL), 1M LiOH (9.7 g in 250 mL of H_2O , 0.23 mol). After stirring overnight, the organic solvents were evaporated and the aqueous residue was extracted with ethyl acetate 35 (2x150 mL). The combined extracts were washed with brine, dried (MgSO_4), and evaporated to afford 10.5 g of 4-methyl-2-methylenepentanoic acid as a colorless oil.

-89-

20B. In a similar manner, the following compounds of formula (Gd) are prepared: 4-phenyl-2-methylenbutanoic acid; 3-cyclohexyl-2-methylenepropanoic acid; 5-phenyl-2-methylenepentanoic acid; 2-methylenepentanoic acid; and 3,3-dimethyl-2-methylenbutanoic acid.

Example 21

Compounds of formula (G)

A mixture of 4-methyl-2-methylenpentanoic acid (5.0 g) and thioacetic acid (25 mL) was heated at 95°C under argon for 3 days. The excess thioacetic acid was evaporated and the residual oil was dissolved in ethyl acetate (40 mL) and extracted with saturated NaHCO₃ (3x40 mL). The combined NaHCO₃ extracts were combined and acidified at 0°C to pH 2 with 1M HCl. The aqueous layer was extracted with CH₂Cl₂ (3x40 mL), the combined organic phases were dried (MgSO₄) and evaporated to give 3.0 g of 4-methyl-2-acetylthiomethyl-pentanoic acid; ¹H NMR (80 MHz, CDCl₃) δ 0.95 (d, J=8.0, 6H), 1.20-1.90 (m, 4H), 2.35 (s, 3H), 2.50-3.20 (m, 3H), 6.7 (br s, 1H).

20

Example 22

Compounds of formula (If)

22A. To a solution of 4-methyl-2-acetylthiomethyl-pentanoic acid (204 mg, 1.0 mmol) in dry DMF (15 mL) containing HOBT (92 mg, 0.6 mmol) and L-leucine-N'-(4-methoxycarbonylphenyl)-carboxamide (0.6 mmol) was added EDCI (345 mg, 1.8 mmol). The solution was stirred overnight at 25°C and then the DMF was removed in vacuo. The residue was dissolved in ethyl acetate (35 mL) and washed with 1M HCl, 1M NaOH, and brine. Drying over MgSO₄ and evaporation afforded a semisolid which was flash chromatographed on silica gel (ethyl acetate 1:petroleum ether 2) to give N-(4-methyl-2-acetylthiomethylpentanoyl)-L-leucine-N'-(4-methoxycarbonylphenyl)carboxamide (190 mg) as a white solid.

35

22B. In a similar manner, the following compounds of formula (If) are prepared:

-90-

- N*- (5-phenyl-2-acetylthiomethylpentanoyl)-L-leucine-
N'-(4-methoxycarbonylphenyl) carboxamide;
- 5 *N*- (4-phenyl-2-acetylthiomethylbutanoyl)-L-leucine-*N'*-(4-
 methoxycarbonylphenyl) carboxamide;
- 10 *N*- (3-phenyl-2-acetylthiomethylpropanoyl)-L-leucine-*N'*-(4-
 methoxycarbonylphenyl) carboxamide;
- 15 *N*- (3-cyclohexyl-2-acetylthiomethylpropanoyl)-L-leucine-*N'*-(4-
 methoxycarbonylphenyl) carboxamide;
- 20 *N*- (2-acetylthiomethylpentanoyl)-L-leucine-*N'*-(4-
 methoxycarbonylphenyl) carboxamide;
- 25 *N*- (5-phenyl-2-acetylthiomethylpentanoyl)-L-leucine-*N'*-(4-
 aminocarbonylphenyl) carboxamide;
- 30 *N*- (4-phenyl-2-acetylthiomethylbutanoyl)-L-leucine-*N'*-(4-
 carboxyphenyl) carboxamide;
- 35 *N*- (3-phenyl-2-acetylthiomethylpropanoyl)-L-leucine-*N'*-(4-
 methysulfonylphenyl) carboxamide;
- 40 *N*- (3-cyclohexyl-2-acetylthiomethylpropanoyl)-L-leucine-*N'*-(4-
 carbamoylphenyl) carboxamide;
- 45 *N*- (2-acetylthiomethylpentanoyl)-L-leucine-*N'*-(4-
 cyanophenyl) carboxamide;
- 50 *N*- (5-phenyl-2-acetylthiomethylpentanoyl)-L-tryptophan-*N'*-(4-
 methoxycarbonylphenyl) carboxamide;
- 55 *N*- (4-phenyl-2-acetylthiomethylbutanoyl)-L-tryptophan-*N'*-(4-
 methoxycarbonylphenyl) carboxamide;
- 60 *N*- (3-phenyl-2-acetylthiomethylpropanoyl)-L-tryptophan-*N'*-(4-
 methoxycarbonylphenyl) carboxamide;
- 65 *N*- (3-cyclohexyl-2-acetylthiomethylpropanoyl)-L-tryptophan-*N'*-(4-
 methoxycarbonylphenyl) carboxamide; and
- 70 *N*- (2-acetylthiomethylpentanoyl)-L-tryptophan-*N'*-(4-
 methoxycarbonylphenyl) carboxamide.

Example 23

Compounds of formula (Ig)

- 23A. To a solution of *N*- (4-methyl-2-
 acetylthiomethylpentanoyl)-L-leucine-*N'*-(4-methoxycarbonyl-
 phenyl) carboxamide (85 mg, 0.19 mmol) in MeOH (8 mL) at 0°C
 was added concentrated HNO₂ (0.4 mL). After stirring at 0°C

-91-

for 5 hours, the methanol was evaporated and ether (30 mL) was added. The ether solution was washed with 0.5 M HCl, brine, and was dried over MgSO₄. Concentration afforded *N*-(4-methyl-2-mercaptopentanoyl)-L-leucine-*N'*-(4-methoxycarbonylphenyl)carboxamide in quantitative yield as a white foam,
5 MS (FAB) 407 (M-H)⁻.

23B. In a similar manner, the following compounds of formula (Ig) are prepared:

- 10 *N*-(5-phenyl-2-mercaptopentanoyl)-L-leucine-
N'-(4-methoxycarbonylphenyl)carboxamide;
N-(4-phenyl-2-mercaptopentanoyl)-L-leucine-*N'*-(4-
methoxycarbonylphenyl)carboxamide;
15 *N*-(3-phenyl-2-mercaptopentanoyl)-L-leucine-*N'*-(4-
methoxycarbonylphenyl)carboxamide;
N-(3-cyclohexyl-2-mercaptopentanoyl)-L-leucine-*N'*-(4-
methoxycarbonylphenyl)carboxamide;
20 *N*-(2-mercaptopentanoyl)-L-leucine-*N'*-(4-
methoxycarbonylphenyl)carboxamide;
N-(5-phenyl-2-mercaptopentanoyl)-L-leucine-
N'-(4-aminocarbonylphenyl)carboxamide;
25 *N*-(4-phenyl-2-mercaptopentanoyl)-L-leucine-*N'*-(4-carboxyphenyl)carboxamide;
N-(3-phenyl-2-mercaptopentanoyl)-L-leucine-*N'*-(4-methylsulfonylphenyl)carboxamide;
N-(3-cyclohexyl-2-mercaptopentanoyl)-L-leucine-*N'*-(4-carbamoylphenyl)carboxamide;
30 *N*-(2-mercaptopentanoyl)-L-leucine-*N'*-(4-cyanophenyl)carboxamide;
N-(5-phenyl-2-mercaptopentanoyl)-L-tryptophan-
N'-(4-methoxycarbonylphenyl)carboxamide;
N-(4-phenyl-2-mercaptopentanoyl)-L-tryptophan-*N'*-(4-
methoxycarbonylphenyl)carboxamide;
35 *N*-(3-phenyl-2-mercaptopentanoyl)-L-tryptophan-*N'*-(4-methoxycarbonylphenyl)carboxamide;
N-(3-cyclohexyl-2-mercaptopentanoyl)-L-tryptophan-*N'*-(4-methoxycarbonylphenyl)carboxamide; and

-92-

N- (2-mercaptopentanoyl)-L-tryptophan-N'-(4-methoxycarbonylphenyl)carboxamide.

Example 24

5 **Formula (Fc²⁴)**

To a stirred solution of 6.48 g (25.0 mmol) of *N*-(4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone in 50 mL of dry THF under argon at -95 °C was added 27.5 mL (27.5 mmol) of 1.0 M sodium hexamethyldisilazide in THF via syringe at a rate to maintain the reaction temperature at less than -75 °C. After 15 min at -80 °C to -95 °C, 5.65 mL (6.83 g, 35 mmol) of *t*-butyl bromoacetate, which had been filtered through basic alumina immediately prior to use, was added via syringe over a 1 min period. The solution was stirred at -90 °C to -60 °C for 2 h, and then partitioned between hexane (100 mL) and dilute aq. NaHSO₄. The organic layer was washed with sat. aq. NaCl containing a little 1 M phosphate buffer (pH 7), dried over Na₂SO₄, and concentrated. The residue was recrystallized from 75 mL of hexane to give 5.56 g (60%) of *N*-(2*R*-(*t*-Butoxycarbonyl)methyl-4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone as pale yellow needles: mp 75-76 °C.
El. Anal. Calc. for C₂₁H₂₇NO₅: C, 67.54; H, 7.29, N, 3.75. Found: C, 67.76; H, 7.34; N, 3.87.

25

Example 25

Formula (Fc²⁵-1) Where R² is Biphenyl

To a solution of 4.75 g (12.7 mmol) of *N*-(2*R*-(*t*-Butoxycarbonyl)-methyl-4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone, 3.73 g (16.0 mmol) of 4-bromobiphenyl, 0.234 g (0.77 mmol) of tri-*o*-tolylphosphine, and 2.22 mL (1.62 g, 16.0 mmol) of triethylamine in 10 mL of anhydrous DMF under argon was added 0.086 g (0.385 mmol) of palladium(II) acetate. The solution was heated at 100 °C for 4 h, cooled to room temperature, diluted with ethyl acetate. The precipitate was removed by filtration, and the filtrate was partitioned between 150 mL of 2:1 ethyl acetate:hexane and 50 mL of pH 7 phosphate buffer (0.5 M) containing a little sodium sulfite.

-93-

The organic layer was washed with 0.2 N aq. sodium bisulfate and brine/pH 7 phosphate buffer, dried over sodium sulfate, and concentrated. The residue was dissolved in 50 mL of ethyl acetate, diluted with 250 mL of isoctane, and seeded with a few crystals of the product. The solid was removed by filtration, and recrystallized from 250 mL of 4:1 isoctane: ethyl acetate to give 4.20 g (63%) of the product, N-(2R-(t-Butoxycarbonyl)methyl-(5-(biphen-4-yl)-4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone as fine white needles: mp 118-119 °C; ¹H NMR (300 MHz, CDCl₃) 7.25-7.60 (m, 14H), 6.47 (d, 1H, J = 16 Hz), 6.25 (dt, 1H, J = 16 and 8 Hz), 4.65-4.70 (m, 1H), 4.34-4.44 (m, 1H), 4.11 (dd, 1H, J = 9 and 2 Hz), 4.01 (t, 1H, J = 8 Hz), 3.33 (dd, 1H, J = 14 and 3 Hz), 2.89 (dd, 1H, J = 17 and 11 Hz), 2.76 (dd, 1H, J = 14 and 10 Hz), 2.40-2.57 (m, 3H), 1.43 (s, 9H).
El. Anal. Calc. for C₃₃H₃₅NO₅: C, 75.40; H, 6.77, N, 2.66. Found: C, 75.17; H, 6.84; N, 2.58.

Example 26

20 **Formula (Fc*-2) Where R² is Biphenyl**

A solution of 5.23 g (10.00 mmol) of N-(2R-(t-butoxycarbonyl)methyl-(5-(biphen-4-yl)-4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone in 50 mL of ethyl acetate was hydrogenated at 1 atm of hydrogen over 500 mg of 10% Pd/C for 2 h at room temperature. The catalyst was removed by filtration through Celite, and the filtrate was concentrated to about 20 mL, then diluted with about 75 mL of isoctane. The solution was seeded with a few crystals of the product, and the mixture was concentrated to about 50 mL, then cooled to -20 °C. Filtration of the precipitate gave 4.91 g (94%) of N-(2R-(t-butoxycarbonyl)methyl-(5-(biphen-4-yl)pentanoyl)-4S-phenylmethyl-2-oxazolidinone as a white powder: mp: 75-76 °C; ¹H NMR (300 MHz, CDCl₃) 7.22-7.59 (m, 14H), 4.61-4.70 (m, 1H), 4.18-4.24 (m, 1H), 4.14 (d, 2H, J = 5 Hz), 3.34 (dd, 1H, J = 13 and 3 Hz), 2.57-2.89 (m, 4H), 2.48 (dd, 1H, J = 13 and 5 Hz), 1.65-1.83 (m, 3H), 1.50-1.60 (m, 1H), 1.42 (s, 9H). El. Anal. Calc. for C₃₃H₃₇NO₅: C, 75.11; H, 7.07, N, 2.65.

-94-

Found: C, 75.34; H, 7.11; N, 2.69.

Example 27

Formula F Where R² is Biphenyl

To a solution of 4.02 g (7.62 mmol) of N-(2R-(t-butoxycarbonyl)-methyl-(5-(biphen-4-yl)pentanoyl)-4S-phenylmethyl-2-oxazolidinone in 60 mL of THF at 0 °C was added 2.8 mL of 30% aq. hydrogen peroxide followed by 8.0 mL of 2 N aq. lithium hydroxide. The mixture was stirred vigorously at 0 °C for 15 min, and then allowed to warm to room temperature. After 2 h, the mixture was cooled to 0 °C, and 20 mL of 2 N aq. sodium sulfite and 30 mL of saturated aq. sodium bicarbonate were added. After 10 min at 0 °C, the mixture was stirred an additional 1 h at room temperature and then poured into 1 M pH 7 phosphate buffer. The aqueous phase was acidified to pH 6 by addition of solid sodium bisulfate, and then the mixture was extracted with 1:1 ethyl acetate:hexane (200 mL). The organic layer was washed with brine, dried over sodium sulfate, and concentrated. The residue was chromatographed on 125 g of silica gel, eluting with 20% to 30% ethyl acetate:hexane containing 0.5% acetic acid. The product-containing fractions were concentrated and then azeotroped several times with toluene to give 2.93 (>100%) of the product, 2R-(t-butoxycarbonyl)methyl-(5-(biphen-4-yl)pentanoic acid, as a thick syrup which slowly solidified upon storage at -20 °C: mp 44-45 °C (after drying solid in vacuo); ¹H NMR (300 MHz, CDCl₃) δ 7.22-7.59 (m, 9H), 2.82-2.90 (m, 1H), 2.59-2.75 (m, 3H), 2.40 (dd, 1H, J = 14 and 5 Hz), 1.55-1.80 (m, 4H), 1.42 (s, 9H).

El. Anal. Calc. for C₂₃H₂₈O₄: C, 74.97; H, 7.66. Found: C, 75.08; H, 7.76.

Example 28

Formula (A-1)

To a solution of 5.00 g (21.6 mmol) of N-(t-butoxycarbonyl)-L-t-leucine and 2.50 g (21.7 mmol) of N-hydroxy-succinimide in 40 mL of acetonitrile at 0 °C was added

-95-

dropwise a solution of 4.12 g (20 mmol) of dicyclohexyl-carbodiimide in 40 mL of acetonitrile. The mixture was stirred at 0 °C to room temperature overnight, and then the mixture was filtered to remove the precipitated
5 dicyclohexylurea. The filtrate was concentrated and the residue was triturated with ethyl acetate/dichloromethane to give 5.06 g (80%) of N-(t-Butoxycarbonyl)-L-t-leucine,
10 N-hydroxysuccinimide ester as a white solid: mp 136-137 °C;
¹H NMR (300 MHz, CDCl₃) δ 5.07 (br d, 1H), 4.43 (d, 1H, J = 10 Hz), 2.84 (s, 4H), 1.46 (s, 9H), 1.10 (s, 9H).

Example 29

Formula (C) Where R³ is t-Butyl, and R⁷ is 4-Pyridine (in place of the illustrated phenyl group)

15 A solution of 2.00 g (6.32 mmol) of N-(t-butoxycarbonyl)-L-t-leucine, N-hydroxysuccinimide ester and 2.98 g (31.6 mmol) of 4-aminopyridine in 20 mL of dioxane was heated at 100 °C for 3 h. The reaction was cooled to room temperature and concentrated. The residue was purified by chromatography on
20 silica gel, eluting with 5% to 10% methanol in dichloromethane, to give 1.06 g (54%) of N-(t-butoxycarbonyl)-L-t-leucine-N'-(pyrid-4-yl)carboxamide as a white solid:
¹H NMR (300 MHz, CDCl₃) δ 8.47 (d, 2H, J = 6 Hz), 8.35 (br s, 1H), 7.50 (d, 2H, J = 6 Hz), 5.23 (broad d, 1H), 4.00 (br d, 1H), 1.44 (s, 9H), 1.05 (s, 9H).
25 FAB-MS ((M+H)⁺ calculated: 308.1974 observed: 308.1970.

Example 30

30A. Formula (D) Where R³ is t-Butyl, and R⁷ is 4-Pyridine (in place of the illustrated phenyl group)

To a solution of 132 mg (0.43 mmol) of N-(t-butoxycarbonyl)-L-t-leucine-N'-(pyrid-4-yl)carboxamide in 2 mL of dichloromethane was added 1 mL of trifluoroacetic acid.
After 1 h at room temperature, the solution was diluted with ca. 5 mL of toluene and concentrated. Repeated dissolution in toluene/dichloromethane/methanol and concentration eventually provided 190 mg (100%) of L-t-leucine-N'-(pyrid-4-

-96-

yl) carboxamide bis(trifluoroacetate) as a white solid: ^1H NMR (300 MHz, DMSO-d₆) δ 11.58 (br s, 1H), 8.68 (d, 2H, J = 6 Hz), 8.35 (br s, 2H), 7.91 (d, 2H, J = 6 Hz), 3.81 (s, 1H), 1.04 (s, 9H).

5 FAB-MS ((M+H)⁺ calculated: 208.1500 observed: 208.1496.

30B. Formula (D) Where R³ is t-Butyl, and R⁷ is 4-(Methyl-thio)phenyl

In a manner analogous to that of part A was prepared L-t-leucine-N'-(4-(methylthio)phenyl)carboxamide: ^1H NMR (300 MHz, CDCl₃) δ 9.02 (s, 1H), 7.49 (d, 2H, J = 6.5 Hz), 7.23 (d, 2H, J = 6.5 Hz), 3.23 (s, 2H), 2.44 (s, 3H), 1.03 (s, 9H).

Example 31

15 **31A. Formula (Ib) Where R² is Biphenyl (and X is propane-1,3-diyl), R³ and R⁸ are t-Butyl, and R⁷ is 4-Pyridine (in place of the illustrated phenyl group)**

To a solution of 357 mg (0.97 mmol) of 2R-(t-butoxycarbonyl)methyl-(5-(biphen-4-yl)pentanoic acid, 422 mg (0.97 mmol) of L-t-leucine-N'-(4-(pyrid-4-yl)carboxamide bis(trifluoroacetate), and 0.50 mL (3.6 mmol) of triethylamine in 5 mL of DMF was added 442 mg (1.00 mmol) of benzotriazol-1-yl-tris-(dimethylamino)phosphonium hexafluorophosphate. After 4 h, the reaction was partitioned between ethyl acetate and water. The aqueous layer was extracted twice with ethyl acetate, and the combined organic layers were washed with water and with brine, dried over sodium sulfate, and concentrated. Purification of the residue by silica gel chromatography, eluting with 25% to 75% ethyl acetate in hexane, gave 360 mg (66%) of N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(pyrid-4-yl)-carboxamide: ^1H NMR (300 MHz, CDCl₃) δ 8.52 (s, 1H), 8.42 (d, 2H, J = 6 Hz), 7.54 (d, 2H, J = 7 Hz), 7.40-7.48 (m, 6 H), 7.32 (t, 1H, J = 7 Hz), 7.13 (d, 2H, J = 8 Hz), 6.62 (d, 1H, J = 9 Hz), 4.35 (d, 1H, J = 9 Hz), 2.58-2.68 (m, 4H), 2.40 (dd, 1H, J = 16 and 3 Hz), 1.40-1.75 (s over m, obscured by H₂O, 13 H), 1.08 (s, 9H).

-97-

31B. Formula (Ib) Varying R⁷

By following the procedure of part A and substituting L-t-leucine-N'-(4-(pyrid-4-yl)carboxamide bis(trifluoroacetate) with the following:

- 5 L-t-leucine-N'-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)-
 carboxamide;
- L-t-leucine-N'-(4-(methylthio)phenyl)carboxamide;
 there are obtained:
- 10 N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-
 leucine -N'-(4-((2-hydroxyethyl)aminosulfonyl)-
 phenyl)carboxamide: mp 89-92 °C; ¹H NMR (300 MHz,
 methanol-d₄) δ 7.80 (s, 4H), 7.51 (d, 2H, J = 7 Hz),
 7.41 (d, 2H, J = 7 Hz), 7.36 (d, 2H, J = 8 Hz), 7.28 (t,
 1H, J = 7 Hz), 7.14 (d, 2H, J = 8 Hz), 4.47 (s, 1H), 3.47
 (t, 2H, J = 6 Hz), 2.85-2.95 (t overlapping m, 3H), 2.52-
 2.62 (m, 3H), 2.32 (dd, 1H, J = 16.5 and 5 Hz), 1.48-1.62
 (m, 4H), 1.41 (s, 9H), 1.09 (s, 9H)
- 15 El. Anal. Calc. for C₃₇H₄₉N₃O₇S: C, 65.37; H, 7.26; N,
 6.18; S, 4.72. Found: C, 65.13; H, 7.33; N, 6.22; S,
 4.63;
- 20 N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-
 leucine-N'-(4-(methylthio)phenyl)carboxamide: ¹H NMR (300
 MHz, CDCl₃) δ 7.79 (s, 1H), 7.53 (d, 2H, J = 7 Hz), 7.31-
 7.44 (m, 7H), 7.19 (d, 2H, J = 9 Hz), 7.13 (d, 2H, J = 8
 Hz), 6.58 (d, 1H, J = 9 Hz), 4.36 (d, 1H, J = 9 Hz),
 2.55-2.67 (m, 4H), 2.34-2.40 (s overlapping m, 4H), 1.38-
 1.75 (s overlapping m, 13 H), 1.07 (s, 9H)
- 25 El. Anal. Calc. for C₃₆H₄₆NO₄S·0.25 H₂O: C, 71.19; H,
 7.72, N, 4.61, S, 5.28. Found: C, 71.20; H, 7.78, N,
 4.58, S, 5.28;

Example 32

- 32A. Formula (Ic) Where R² is Biphenyl (and X is propane-1,3-diyl), R³ is t-Butyl, and R⁷ is 4-Pyridine (in place of the illustrated phenyl group)

To a solution of 360 mg (0.64 mmol) of N-(2R-(t-butoxycarbonyl)-methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-

-98-

N'- (pyrid-4-yl)carboxamide in 4 mL of dichloromethane was added 2 mL of trifluoroacetic acid. After 1 h at room temperature, the solution was diluted with toluene and concentrated. The residue was dissolved in ethyl acetate (15 mL) and washed with 0.5 M pH 4 citrate buffer (2 x 15 mL).
5 The combined aq. layers were extracted with ethyl acetate (2 x 15 mL), and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The residue was triturated with ethyl acetate/hexane to give 230 mg (71%) of N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(pyridin-4-yl)carboxamide as a white solid:
10 mp 198-201 °C; ¹H NMR (300 MHz, MeOH-d4) δ 8.32 (d, 2H, J = 7 Hz), 7.64 (d, 2H, J = 5 Hz), 7.45 (d, 2H, J = 7 Hz), 7.24-7.38 (m, 7H), 7.10 (d, 2H, J = 8 Hz), 4.42 (s, 1H), 2.85-3.00 (m, 1H), 2.33-2.65 (m, 4H), 1.40-1.62 (m, 4H), 1.02 (s, 9H).
15 El. Anal. Calc. for C₃₀H₃₆N₃O₄•0.5 H₂O•0.5 ethyl acetate (solvate): C, 69.29; H, 7.27, N, 7.58. Found: C, 69.46; H, 7.09; N, 7.55.

20 **32B. Formula (Ic) Varying R'**

By following the procedure of part A and substituting N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(pyrid-4-yl)carboxamide with the following:

N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine -N'-(4-((2-hydroxyethyl)aminosulfonyl)-phenyl)carboxamide;

N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(4R/S-(methylsulfinyl)phenyl)carboxamide;

N-(2R-(t-butoxycarbonyl)methyl-5-(fluoren-2-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;

N-(2R-(t-butoxycarbonyl)methyl-5-(7-(glycyl)aminofluoren-2-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;

N-(2R-(t-butoxycarbonyl)methyl-5-(4-(pyrid-4-yl)phenyl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;

N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-β-

-99-

- hydroxyvaline-N'-(phenyl)carboxamide;
N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(4-(methylsulfonyl)phenyl)carboxamide;
N-(2R-(t-butoxycarbonyl)methyl-5-(4-(2-hydroxyethyl)phenyl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;
N-(2R-(t-butoxycarbonyl)methyl-5-(4'-hydroxybiphen-4-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;
10 N-(2R-(t-butoxycarbonyl)methyl-5-(4'-cyanobiphen-4-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;
N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;
15 N-(2R-(t-butoxycarbonyl)methyl-5-(4'-(2-aminoethoxy)biphen-4-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;
N-(2R-(t-butoxycarbonyl)methyl-5-(4-(pyridin-4-yl)phenyl)pentanoyl)-L-cyclohexylglycine-N'-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)carboxamide;
20 N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-threonine-N'-(4S-(methylsulfinyl)phenyl)carboxamide;
N-(2R-(t-butoxycarbonyl)methyl-5-(2-fluorobiphen-4-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;
25 N-(2R-(t-butoxycarbonyl)methyl-4-((biphen-4-yl)thio)butanoyl)-L-t-leucine-N'-(pyridin-4-yl)carboxamide;
N-(2R-(t-butoxycarbonyl)methyl-5-(4-(2-aminopyridin-5-yl)phenyl)pentanoyl)-L-threonine-N'-(4S-(methylsulfinyl)phenyl)carboxamide;
30 N-(2R-(t-butoxycarbonyl)methyl-5-(2-hydroxybiphen-4-yl)pentanoyl)-L-threonine-N'-(4S-(methylsulfinyl)phenyl)carboxamide;
N-(2R-(t-butoxycarbonyl)methyl-5-(4'-cyanobiphen-4-yl)pentanoyl)-L-(trans-4-hydroxycyclohexyl)glycine-N'-(4S-(methylsulfinyl)phenyl)carboxamide;
35 N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-(4-

-100-

hydroxytetrahydropyran-4-yl)glycine-N'-(4S-(methylsulfinyl)phenyl)carboxamide; and

N-(2R-(t-butoxycarbonyl)methyl-5-(2R/S-hydroxy-3,3,3-trifluoropropyl)phenyl)pentanoyl)-L-(cyclohexyl)glycine-N'-(4S-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)-carboxamide,

there are obtained:

N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)carboxamide:

¹H NMR (300 MHz, MeOH-d4) δ 7.80 (s, 4H), 7.50 (d, 2H, J = 7 Hz), 7.25-7.42 (m, 5H), 7.15 (d, 1H, J = 8 Hz), 4.47 (s, 1H), 3.47 (t, 2H, J = 6 Hz), 2.89-3.00 (m, 1H), 2.87 (t, 2H, J = 6 Hz), 2.49-2.70 (m, 3H), 2.39 (dd, 1H, J = 16 and 5 Hz), 1.46-1.67 (m, 4H), 1.07 (s, 9H);

El. Anal. Calc. for C₃₃H₄₁N₃O₇S°0.5 H₂O: C, 62.64; H, 6.69; N, 6.64; S, 5.07. Found: C, 62.61; H, 6.80; N, 6.31; S, 4.97;

N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(4R/S-(methylsulfinyl)phenyl)carboxamide:

¹H NMR (300 MHz, MeOH-d4) δ 8.03 (d, 1H, J = 9 Hz), 7.76 (d, 2H, J = 9 Hz), 7.57 (dd, 2H, J = 9 and 2 Hz), 7.44 (d, 2H, J = 7 Hz), 7.36 (t, 2H, J = 7 Hz), 7.23-7.28 (m, 3H), 7.10 (d, 2H, J = 8 Hz), 4.45 (d, 1H, J = 9 Hz), 2.85-2.98 (m, 1H, J = 8 Hz), 2.44-2.64 (m, 7H), 2.35 (dd, 1H, J = 16 and 5 Hz), 1.43-1.62 (m, 4H), 1.03 (s, 9H);

N-(2R-carboxymethyl-5-(fluoren-2-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide: mp 188-190 °C;

¹H NMR (300 MHz, CDCl₃) δ 12.06 (s, 1H), 10.30 (s, 1H), 8.19 (d, 1H, J = 8 Hz), 7.86 (d, 2H, J = 9 Hz), 7.68-7.77 (m, 3H), 7.62 (d, 1H, 8 Hz), 7.48 (d, 1H, J = 7 Hz), 7.20-7.35 (m, 3H), 7.10 (d, 1H, J = 7 Hz), 4.48 (m, 1H), 3.76 (s, 3H), 3.70 (s, 2H), 2.15-2.75 (m, 5H), 1.35-1.75 (m, 5H), 0.90-0.99 (m, 4H)

El. Anal. Calc. for C₃₄H₃₈N₂O₆: C, 71.56; H, 6.71, N, 4.91.

Found: C, 71.51; H, 6.97; N, 4.84;

N-(2R-carboxymethyl-5-(7-(glycyl)aminofluoren-2-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide:

mp 222-224 °C; FAB-MS (M+H)⁺ calculated for C₄₀H₅₁N₄O₇:

699.3758; observed: 699.3770;

-101-

N-(2R-carboxymethyl-5-(4-(pyrid-4-yl)phenyl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide:

¹H NMR (300 MHz, d₄-MeOH) δ 8.52 (d, 2H, J = 5.5 Hz), 7.92 (d, 2H, J = 9.19 Hz), 7.67 (d, 2H, J = 8.82 Hz), 7.58 (d, 2H, J = 6.25 Hz), 7.46 (d, 2H, J = 8.45 Hz), 7.20 (d, 2H, J = 8.46 Hz), 4.6-4.4 (m, 1H), 3.84 (s, 3H), 2.83-2.59 (m, 4H), 2.38 (dd, 1H, J = 16.7 and 5 Hz), 1.71-1.57 (m, 7H), 0.96 (dd, 6H, J = 9.92 and 6.3 Hz);

N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-β-hydroxyvaline-N'-(phenyl)carboxamide:

¹H NMR (300 MHz, CDCl₃) δ 8.73 (s, 1H), 8.07 (d, 1H, J = 8.09 Hz), 7.52-7.25 (m, 11H), 7.10 (t, 1H, J = 7.54 Hz), 6.97 (d, 2H, J = 8.08 Hz), 4.41 (d, 1H, J = 8.45 Hz), 3.02-3.00 (m, 1H), 2.75 (dd, 1H, J = 16.55 and 8.45 Hz), 2.53-2.51 (m, 2H), 2.44 (dd, 1H, J = 17.1 and 4.6 Hz), 1.85-1.47 (m, 4H), 1.45 (s, 3H), 1.21 (s, 3H)

El. Anal. Calc. for C₃H₃N₄O₄: C, 71.69; H, 6.82; N, 5.57.

Found: C, 71.65; H, 6.86; N, 5.53;

N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(4-(methyl-sulfonyl)phenyl)carboxamide:

¹H NMR (300 MHz, MeOH-d4) δ 8.10 (d, 1H, J = 9 Hz), 7.84 (s, 4H), 7.47 (d, 2H, J = 8 Hz), 7.25-7.40 (m, 5H), 7.13 (d, 2H, J = 8 Hz), 4.48 (d, 1H, J = 9 Hz), 2.95 (s, 3H), 2.44-2.70 (m, 4H), 2.36 (dd, 1H, J = 16 and 5 Hz), 1.47-1.63 (m, 4H), 1.06 (s, 9H);

N-(2R-carboxymethyl-5-(4-(2-hydroxyethyl)phenyl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide:

¹H NMR (300 MHz, MeOH-d4) δ 7.91 (d, 2H, J = 9 Hz), 7.64 (d, 2H, J = 9 Hz), 6.96 (s, 4H), 4.47-4.51 (m, 1H), 3.84 (s, 3H), 3.63 (t, 2H, J = 7 Hz), 2.68 (t, 2H, J = 7 Hz), 2.46-2.75 (m, 4H), 2.37 (dd, 1H, J = 16 and 5 Hz), 1.51-1.73 (m, 7H), 0.93 and 0.89 (2d, 6H, J = 7 Hz);

N-(2R-carboxymethyl-5-(4'-hydroxybiphen-4-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide:

mp 195-197 °C; FAB-MS (M+H) expected: 575.2757; observed: 595.2750;

N-(2R-carboxymethyl-5-(4'-cyanobiphen-4-yl)pentanoyl)-L-

-102-

leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide:

¹H NMR (300 MHz, MeOH-d4) δ 10.02s, 1H), 8.37 (d, 1H, J=7 Hz), 7.87 (d, 2H, J=8 Hz), 7.71 (d, 2H, J=8.5 Hz), 7.64 (d, 4H, J=9 Hz), 7.35 (d, 2H, J= 8 Hz), 7.16 (d, 2H, J= 8 Hz), 4.50-4.53 (m, 1H, 3.81 (s, 3H), 2.49-2.78 (m, 4H), 2.35 (dd, 1H, J= 16 and 5 Hz), 1.46-1.72 (m, 7H); 0.88 and 0.90 (2d, 2H, J= 7 Hz); N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide:

mp 191-193 °C;

10 N-(2R-carboxymethyl-5-(4'-(2-aminoethoxy)biphen-4-yl)-pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)-carboxamide:

FAB-MS (M+H)⁺ calc. 618.3179; found: 618.3189;

15 N-(2R-carboxymethyl-5-(4-(pyridin-4-yl)phenyl)pentanoyl)-L-cyclohexylglycine-N'-(4-((2-hydroxyethyl)aminosulfonyl)-phenyl)carboxamide;

N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-threonine-N'-(4S-(methylsulfinyl)phenyl)carboxamide;

20 N-(2R-carboxymethyl-5-(2-fluorobiphen-4-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide;

N-(2R-carboxymethyl-4-((biphen-4-yl)thio)butanoyl)-L-t-leucine-N'-(pyridin-4-yl)carboxamide;

25 N-(2R-carboxymethyl-5-(4-(2-aminopyridin-5-yl)phenyl)-pentanoyl)-L-threonine-N'-(4S-(methylsulfinyl)phenyl)-carboxamide;

N-(2R-carboxymethyl-5-(2-hydroxybiphen-4-yl)pentanoyl)-L-threonine-N'-(4S-(methylsulfinyl)phenyl)carboxamide;

30 N-(2R-carboxymethyl-5-(4'-cyanobiphen-4-yl)pentanoyl)-L-(trans-4-hydroxycyclohexyl)glycine-N'-(4S-(methylsulfinyl)phenyl)carboxamide;

N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-(4-hydroxytetrahydropyran-4-yl)glycine-N'-(4S-(methylsulfinyl)phenyl)carboxamide; and

35 N-(2R-carboxymethyl-5-(2R/S-hydroxy-3,3,3-trifluoropropyl)-phenyl)pentanoyl)-L-(cyclohexyl)glycine-N'-(4S-(4-((2-hydroxyethyl)aminosulfonyl) phenyl)carboxamide.

-103-

Example 33

33A. Formula (Ie) Where R² is Biphenyl (and X is propane-1,3-diyl), R³ is t-Butyl, and R⁷ is 4-Pyridine (in place of the illustrated phenyl group)

5 To a solution of 127.4 mg (0.200 mmol) of N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(pyridin-4-yl)carboxamide and 40 μ L of N-methyl morpholine in 1.0 mL of DMF at room temperature was added 115 mg (0.26 mmol) of benzotriazol-1-yl-tris(dimethylamino)phosphonium hexafluorophosphate. After 15 min, 42 mg (0.60 mmol) of hydroxylamine hydrochloride was added in one portion, followed by addition of an additional 70 μ L of N-methyl morpholine. The mixture was stirred for 24 h at room temperature, then partitioned between 30 mL of ethyl acetate and 25 mL of 0.5 M aq. sodium bicarbonate. The organic layer was washed with additional aq. sodium bicarbonate and with brine/pH 7 buffer, dried over sodium sulfate, and concentrated. Recrystallization from ethyl acetate provided 42.2 mg of N-(2R-(N-hydroxycarbamoyl)-methyl-5-(biphen-4-yl)pentanoyl-L-t-leucine-N'-(pyrid-4-yl)-carboxamide. Concentration of the filtrate and purification by radial chromatography (1 mm plate, 5% to 10% ethanol:dichloromethane) provided, after recrystallization from 2:1 ethyl acetate:hexane, an additional 21.0 mg of product. Total yield was 63.1 mg (61%) of N-(2R-(N-hydroxycarbamoyl)methyl-5-(biphen-4-yl)pentanoyl-L-t-leucine-N'-(pyrid-4-yl)carboxamide as a white powder: ¹H NMR (300 MHz, DMSO-d6) δ 10.45 (s, 1H), 10.34 (s, 1H), 8.68 (s, 1H), 8.38 (d, 2H, J = 7 Hz), 8.04 (d, 1 H, J = 9 Hz), 7.25-7.60 (m, 9H), 7.12 (d, 2H, J = 7 Hz), 4.39 (d, 1H, J = 9 Hz), 2.86-2.97 (m, 1H), 2.36-2.60 (m, 2H, obscured by DMSO-d5 resonance), 2.14 (dd, 1H, J = 15 and 7 Hz), 2.02 (dd, 1H, J = 15 and 8 Hz), 1.30-1.53 (m, 4H), 0.94 (s, 9H).

El. Anal. Calc. for C₃₀H₃₆N₄O₄·0.25 H₂O: C, 69.14; H, 7.06, N, 10.75. Found: C, 69.15; H, 7.23; N, 10.56.

35

33B. Formula (Ie) Varying R², R³, and R⁷

By following the procedure of part A and substituting N-

-104-

- (2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-
 (pyridin-4-yl)carboxamide with the following:
- N- (2R- (carboxymethyl-5- (biphen-4-yl) pentanoyl) -L-threonine-N' -
 (4S- (methylsulfinyl) phenyl) carboxamide;
- 5 N- (2R- (carboxymethyl-5- (4- (pyridin-4-yl) phenyl) pentanoyl) -L-
 (β-hydroxy) valine-N' - (4S-
 methylsulfinyl) phenylcarboxamide; and
- N- (2R- (carboxymethyl-4-methylpentanoyl) -L-t-leucine-N' -
 (pyridin-4-yl) carboxamide;
- 10 N- (2R- (carboxymethyl- (5- (4- (2S-
 hydroxypropyl) phenyl) pentanoyl) -L- (trans-4-
 hydroxycyclohexyl) glycine-N' - (pyridin-4-yl) carboxamide;
- N- (2R- (carboxymethyl-5- (4- (2-methylthiazol-
 4yl) phenyl) pentanoyl) - L- (β-hydroxy) valine-N' - (pyridin-
 15 4yl) carboxamide; and
- N- (2R- (carboxymethyl-5- (4- (2R/S-hydroxy-3,3,3-trifluoropropyl)
 phenyl) pentanoyl) -L- (β-hydroxy) valine-N' (pyridin-4-
 yl) carboxamide,
- there are obtained:
- 20 N- (2R- (N-hydroxycarbamoyl) methyl-5- (biphen-4-yl) pentanoyl) -L-
 threonine-N' - (4S- (methylsulfinyl) phenyl) carboxamide;
- N- (2R- (N-hydroxycarbamoyl) methyl-5- (4- (pyridin-4-
 yl) phenyl) pentanoyl) -L- (β-hydroxy) valine-N' - (4S-
 methylsulfinyl) phenylcarboxamide;
- 25 N- (2R- (N-hydroxycarbamoyl) methyl-4-methylpentanoyl) -L-t-
 leucine-N' - (pyridin-4-yl) carboxamide;
- N- (2R- (N-hydroxycarbamoyl) methyl- (5- (4- (2S-
 hydroxypropyl) phenyl) pentanoyl) -L- (trans-4-
 hydroxycyclohexyl) glycine-N' - (pyridin-
 30 4-yl) carboxamide;
- N- (2R- (N-hydroxycarbamoyl) methyl-5- (4- (2-methylthiazol-4-
 yl) phenyl) pentanoyl) -L- (β-hydroxy) valine-N' - (pyridin-4-
 yl) carboxamide;
- N- (2R- (N-hydroxycarbamoyl) methyl-5- (4- (2R/S-hydroxy-3,3,3-
 35 trifluoropropyl) phenyl) pentanoyl) -L- (β-hydroxy) valine-
 N' (pyridin-4-yl) carboxamide.
- N- (2R- (N-hydroxycarbamoyl) methyl (5- (4- (2S-hydroxypropyl) -

-105-

phenyl)pentanoyl)-L-(trans-4-hydroxycyclohexyl)glycine-N'-(pyridin-4-yl)carboxamide;
N-(2R-(N-hydroxycarbamoyl)methyl-5-(4-(2-methylthiazol-4-yl)phenyl)pentanoyl)-L-(β -hydroxy)valine-N'-(pyridin-4-yl)carboxamide; and
5 N-(2R-(N-hydroxycarbamoyl)methyl-5-(4-(2R/S-hydroxy-3,3,3-trifluoropropyl)phenyl)pentanoyl)-L-(β -hydroxy)valine-N'-(pyridin-4-yl)carboxamide.

10

Example 34

34A. Formula (C) Where R³ is t-Butyl, and R⁴ and R⁵ are H
A solution of 2.00 g (6.32 mmol) of the N-hydroxysuccinimide ester of N-(t-butoxycarbonyl)-L-t-leucine in 9 mL of distilled aniline was stirred and heated at 100 °C
15 for 30 min. The mixture was allowed to cool to room temperature, and diluted with 40 mL of ethyl acetate. The solution was washed with 4 x 50 mL of 1 N aq. sodium bisulfate, and the combined aqueous layers were extracted with 25 mL of ethyl acetate. The combined organic layers were
20 washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by chromatography on silica gel (2% to 10% ethyl acetate in dichloromethane) to give 1.36 g (74%) of N-(t-butoxycarbonyl)-L-t-leucine-N'-phenylcarboxamide as a white solid: ¹H NMR (300 MHz, CDCl₃) δ 7.49 (d, 2H, J = 8 Hz), 7.31 (t, 2H, J = 8 Hz), 7.11 (t, 1H, J = 7 Hz), 5.30-5.36 (m, 1H), 3.95 (d, 1H, J = 9 Hz), 1.44 (s, 9H), 1.07 (s, 9H).

-106-

34B. Formula (C) Where R³ is t-Butyl, R⁴ is 4-Methylthio, and R⁵ is H

In a manner analogous to that of part A was prepared N-(t-butoxycarbonyl)-L-t-leucine-N'-(4-(methylthio)phenyl)-carboxamide: ¹H NMR (300 MHz, CDCl₃) δ 7.65 (br s, 1H), 7.42 (d, 2H, J = 8.5 Hz), 7.21 (d, 2H, J = 8.5 Hz), 5.32 (br d, 1H), 3.95 (d, 1H, J = 8.5 Hz), 2.45 (s, 3H), 1.44 (s, 9H), 1.06 (s, 9H).

El. Anal. Calc. for C₁₈H₂₃N₂O₃S: C, 61.33; H, 8.01, N, 7.95, S, 9.09. Found: C, 61.34; H, 8.06; N, 8.00, S, 9.18.

Example 35

Formula (C) Having A Trifluoroacetyl Protecting Group, Where R³ is t-Butyl, and R⁴ and R⁵ are H

To a solution of 1.36 g (4.4 mmol) of N-(t-butoxycarbonyl)-L-t-leucine-N'-phenylcarboxamide in 10 mL of dichloromethane was added 5 mL of trifluoroacetic acid. After 45 min at room temperature, the solution was diluted with toluene and concentrated. The residue was twice more concentrated from toluene to remove excess trifluoroacetic acid, then dried under vacuum (ca. 1 mm Hg). The residue was then dissolved in 15 mL of dichloromethane and treated successively with pyridine (0.90 mL, 11 mmol) and trifluoroacetic anhydride (0.70 mL, 4.84 mmol). After 30 min, the mixture was partitioned between dichloromethane (25 mL) and 1 N aq. sodium bisulfate. The organic layer was washed with additional aq. sodium bisulfate, brine, dried over sodium sulfate, and concentrated to give 1.22 g (91%) of N-(trifluoroacetyl)-L-t-leucine-N'-phenylcarboxamide as a white solid: mp 201-203 °C, ¹H NMR (300 MHz, CDCl₃) δ 7.50 (d, 2H, J = 8 Hz), 7.36 (t, 2H, J = 8 Hz), 7.17 (t, 1H, J = 7 Hz), 4.43 (d, 1H, J = 9 Hz), 1.10 (s, 9H).

El. Anal. Calc. for C₁₄H₁₇F₃N₂O₂: C, 55.62; H, 5.67, N, 9.27. Found: C, 55.57; H, 5.60; N, 9.18.

-107-

Example 36

Formula (C) Having A Trifluoroacetyl Protecting Group, Where R³ is t-Butyl, R⁴ is 4-(2-Hydroxyethyl)aminosulfonyl, and R⁵ is H

To a solution of 250 mg (0.83 mmol) of N-(trifluoroacetyl)-L-t-leucine-N'-phenylcarboxamide in 5 mL of chloroform was added 0.4 mL (6 mmol) of chlorosulfonic acid. The mixture was heated to reflux for 35 min and then cooled to 0 °C and diluted with ethyl acetate. Ethanolamine (1.5 mL) was added and the mixture was stirred at 0 °C for 15 min. The mixture was partitioned between water and ethyl acetate, and the organic layer was washed with 1 N aq. sodium bisulfate, dried over sodium sulfate, and concentrated to provide 140 mg (40%) of N-(trifluoroacetyl)-L-t-leucine-N'-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)carboxamide: ¹H NMR (300 MHz, CDCl₃) δ 7.93 (s, 1H), 7.84 (d, 2H, J = 9 Hz), 7.67 (d, 2H, J = 9 Hz), 7.15 (br d, 1H), 4.90 (br t, 1H), 4.49 (d, 1H, J = 9 Hz), 3.70 (t, 2H, J = 5 Hz), 3.11 (q, 2H, J = 5 Hz), 1.12 (s, 9H).

20

Example 37

Formula (D) Where R³ is t-Butyl, R⁴ is 4-(2-Hydroxyethyl)-aminosulfonyl, and R⁵ is H

To a solution of 257 mg (0.6 mmol) of N-(trifluoroacetyl)-L-t-leucine-N'-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)-carboxamide in 8 mL of ethanol was added 227 mg (6 mmol) of sodium borohydride. The mixture was heated to 55 °C for 15 min, allowed to cool to room temperature, and quenched with 10% ammonium hydroxide in methanol. After 20 h at room temperature, the mixture was filtered and the filtrate was absorbed onto silica gel. Chromatography (dichloromethane to 90:9:1 dichloromethane:methanol:ammonium hydroxide) gave 110 mg (56%) of L-t-leucine-N'-(4-((2-hydroxyethyl)aminosulfonyl)phenyl)carboxamide, as an oil: ¹H NMR (300 MHz, CDCl₃) δ 9.48 (br s, 1H), 7.81 (d, 2H, J = 9 Hz), 7.72 (d, 2H, J = 9 Hz), 3.67 (t, 2H, J = 5 Hz), 3.30 (s, 1H), 3.08 (q, 2H, J = 5 Hz), 1.06 (s, 9H). FAB-MS (M+H⁺): expected 330.1488 observed 330.1480

-108-

Example 38

Formula (Ib) Where R² is Biphenyl (X is propane-1,3-diyl), R³ is t-Butyl, R⁴ is 4R/S-Methylsulfinyl, and R⁵ is H

To a solution of 60.3 mg (0.100 mmol) of N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(4-(methylthio)phenyl)carboxamide in 2 mL of dichloromethane at -78 °C was added a solution of 26 mg (0.15 mmol) of m-chloroperbenzoic acid in 1 mL of dichloromethane. The reaction was stirred at -78 °C for 50 min, and then 0.2 mL of dimethyl sulfide was added. The mixture was allowed to warm to room temperature, and then partitioned between dichloromethane and sat. aq. sodium bicarbonate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated to give 59.6 mg (96%) of N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(4R/S-(methylsulfinyl)phenyl)carboxamide, as a white solid: ¹H NMR (300 MHz, CDCl₃) δ 8.46 (br s, 1H), 7.32-7.68 (m, 11 H), 7.14 (d, 2H, J = 8 Hz), 6.63 (d, 1H, J = 9Hz), 4.42 (d, 1H, J = 9 Hz), 2.36-2.72 (m, 8H), 1.42-1.76 (s overlapping m, 13 H), 1.09 (s, 9H).

Example 39

Formula (F")

To a solution of 2.779 g (7.50 mmol) of N-(2R-(t-butoxycarbonyl)-methyl-4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone in 30 mL of THF at 0 °C was added 2.55 mL (22.5 mmol) of 30% aq. hydrogen peroxide, followed by the addition of 7.5 mL (15 mmol) of 2.0 N aq. lithium hydroxide. The mixture was stirred for 2 h at 0 °C and for 0.5 h at room temperature. After the mixture was recooled to 0 °C, 15 mL of 2 M aq. sodium sulfite and 23 mL of sat. aq. sodium bicarbonate were added. The mixture was stirred an additional 30 min at 0 °C, and then most of the THF was removed by concentration in vacuo. The residue was partitioned between CH₂Cl₂ and H₂O, and the aq. layer was extracted with additional CH₂Cl₂. The combined organic layers were extracted with aq. sodium bicarbonate, and then the combined aq. layers were

-109-

acidified to pH 2 with sodium bisulfate. The resulting mixture was extracted twice with ethyl acetate, and the combined organic layers were washed with sat. aq. NaCl, diluted with 0.25 volume of hexane, dried over Na₂SO₄, and 5 concentrated to provide 1.47 g (92%) of 2R-(t-butoxycarbonyl)-methyl-4-pentenoic acid as a colorless oil: ¹H NMR (300 MHz, CDCl₃) δ 5.68-5.82 (m, 1H), 5.07-5.15 (m, 2H), 2.86-2.96 (m, 1H), 2.60 (dd, 1H, J = 18 and 10 Hz), 2.25-2.52 (m, 3H), 1.43 (s, 9H).

10

Example 40

Formula (D'-1) Where R³ is t-Butyl and R⁷ is 4-(methoxycarbonyl)phenyl

In a manner analogous to Example 31, substituting 2R-(t-butoxycarbonyl)methyl-(5-(biphen-4-yl)pentanoic acid with 2R-(t-Butoxycarbonyl)methyl-4-pentenoic acid, and substituting L-t-leucine-N'-(4-(pyrid-4-yl)carboxamide with L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide, there was prepared N-(2R-(t-butoxycarbonyl)methyl-4-pentenoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide: mp 118-119 °C (cyclohexane); ¹H NMR (300 MHz, CDCl₃) δ 8.96 (s, 1H), 7.95 (d, 2H, J = 9Hz), 7.57 (d, 2H, J = 9 Hz), 6.32 (d, 1H, J = 7Hz), 5.63-5.77 (m, 1H), 4.95-5.08 (m, 2H), 4.52-4.60 (m, 1H), 3.88 (s, 3H), 2.36-2.77 (m, 4H), 2.14-2.27 (m, 1H), 1.60-1.87 (m, 3H), 1.45 (s, 9H), 0.97 (d, 3H, J = 7 Hz), 0.91 (d, 3H, J = 7 Hz).

El. Anal. Calc. for C₂₅H₃₆N₂O₆: C, 65.20; H, 7.88, N, 6.08.

Found: C, 65.04; H, 7.80; N, 6.06.

30

Example 41

41A. Formula (D'-2) Where R³ is Fluoren-2-yl (X is propane-1,3-diyl), R³ is t-Butyl, and R⁷ is 4-(methoxycarbonyl)phenyl

To a solution of 167 mg (0.36 mmol) of N-(2R-(t-butoxycarbonyl)-methyl-4-pentenoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide, 108 mg (0.44 mmol) of 2-bromofluorene, 21 mg (0.07 mmol) of tri-o-tolylphosphine, and 69 μL (50 mg, 0.50 mmol) of triethylamine in 1.0 mL of DMF

-110-

under argon was added 8.0 mg (0.035 mmol) of palladium diacetate. The solution was heated at 100 °C for 2 h, cooled to room temperature, and then partitioned between 3:1 ethyl acetate:hexane and water. The organic layer was washed with 5 1N aq. sodium bisulfate and with brine/pH 7 buffer, dried over sodium sulfate, and concentrated. Purification by flash chromatography (20 g silica, 5% to 10% t-butyl methyl ether in dichloromethane) gave 185 mg (82%) of the product as a solid containing trace impurities by TLC. Recrystallization from t- 10 butyl methyl ether/isooctane provided 115 mg (51%) of N-(2R-(t-butoxycarbonyl)methyl-5-(fluoren-2-yl)-4E-pentenoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide as fine white needles: mp 189-192 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.32 (s, 1H), 7.79 (d, 2H, J = 8 Hz), 7.72 (d, 1H, J = 8 Hz), 15 7.57 (d, 1H, J = 8 Hz), 7.45-7.53 (m, 3 H), 7.36 (t, 1H, J = 7.5 Hz), 7.25-7.32 (m, 2H), 7.17 (d, 1H, J = 7 Hz), 4.52-4.60 (m, 1H), 3.75 (s, 5H), 2.33-2.87 (m, 5H), 1.60-1.90 (m, 3H), 1.45 (s, 9H), 0.92 (apparent t, 6H).
El. Anal. Calc. for C₃₈H₄₄N₂O₆·0.5 H₂O: C, 72.01; H, 7.16, N, 20 4.42. Found: C, 71.87; H, 7.07; N, 4.32.

41B. Formula (D'-2) Where R² is 7-(N-(benzyloxycarbonyl)-glycyl)aminofluoren-2-yl (X is propane-1,3-diyl), R³ is t-Butyl, and R⁷ is 4-(methoxycarbonyl)phenyl
25 To a solution of 600 mg (2.31 mmol) of 2-amino-7-bromofluorene and 483 mg (2.31 mmol) of N-(benzyloxycarbonyl)-glycine in 10 mL of anhydrous pyridine was added 442 mg (2.31 mmol) of EDC hydrochloride. The reaction was heated at 60 °C for 4 days, and then the solution was concentrated. The residue was partitioned between ethyl acetate and 1 N aq. 30 hydrochloric acid, and the organic layer was washed with sat. aq. sodium bicarbonate and with brine, dried over magnesium sulfate, and concentrated to give 813 mg (78%) of N-(benzyloxycarbonyl)glycine-N'-(7-bromofluoren-2-yl)carboxamide as a tan solid: mp 194-195 °C.
35 By following the procedure of part A and substituting 2-bromofluorene with N-(benzyloxycarbonyl)glycine-N'-(7-

-111-

bromofluoren-2-yl)carboxamide, there is obtained N-(2R-(t-butoxycarbonyl)methyl-5-(7-(N-(benzyloxy-carbonyl)glycyl)-aminofluoren-2-yl)-4E-pentenoyl)-L-leucine-N'-(4-methoxycarbonyl)phenyl)carboxamide: mp 213-214 °C.

5 FAB-MS (M+Cs)⁺ calculated for C₄₈H₅₄N₄O₉°Cs: 963.2945; observed: 963.2960.

El. Anal. Calc. for C₄₇H₅₄N₄O₉: C, 69.40; H, 6.51, N, 6.75. Found: C, 69.47; H, 6.51; N, 6.70.

10 41C. Formula (D'-2) Where R² is 4-(pyrid-4-yl)phenyl (X is propane-1,3-diyl), R³ is t-Butyl, and R⁷ is 4-(methoxycarbonyl)phenyl

Aq. 2 M sodium carbonate (3 mL) was added to a suspension of 400 mg (2.0 mmol) of 4-bromopyridine in 2 mL of benzene to give 2 clear phases, and argon was bubbled through the mixture for a few minutes before added 115 mg (0.10 mmol) of palladium tetrakis(triphenylphosphine). To the resulting mixture was added a solution of 200 mg (1.00 mmol) of 4-bromophenylboronic acid in 1 mL of ethanol, and the mixture was heated at reflux for 4 h. After cooling to room temperature, the mixture was partitioned between ethyl acetate (25 mL) and water (25 mL). The organic layer was dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography, eluting with 25% to 50% ethyl acetate in hexane, gave 193 mg (82%) of 4-(4-bromophenyl)-pyridine as a white solid: mp 124-126 °C.

By following the procedure of part A and substituting 2-bromofluorene with 4-(4-bromophenyl)pyridine, there is obtained N-(2R-(t-butoxycarbonyl)methyl-5-(4-(pyrid-4-yl)-phenyl)-4E-pentenoyl)-L-leucine-N'-(4-(methoxycarbonyl)-phenyl)carboxamide: ¹H NMR (300 MHz, CDCl₃) δ 8.80 (s, 1H), 8.64 (d, 2H, J = 6 Hz), 7.85 (d, 2H, J = 8.5 Hz), 7.49 (d, 2H, J = 9 Hz), 7.45 (d, 2H, J = 6 Hz), 7.19 (d, 2H, J = 8 Hz), 6.40 (d, 1H, J = 16 Hz), 6.33 (d, 1H, J = 8 Hz), 6.09-6.17 (m, 1H), 4.54-4.57 (m, 1H), 3.80 (s, 3H), 2.38-2.81 (m, 5H), 1.48-1.84 (m, 3H), 1.44 (s, 9H), 0.90 and 0.94 (2 d, 6H, J = 7 Hz).

-112-

Example 42

42A. Formula (Ib') Where R² is Fluoren-2-yl (X is propane-1,3-diyl), R³ is t-Butyl, and R⁷ is 4-(methoxycarbonyl)phenyl

5 A solution of 111 mg (0.177 mmol) of N-(2R-(t-butoxycarbonyl)methyl-5-(fluoren-2-yl)-4-pentenoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide in 7 mL of 4:3 ethyl acetate:ethanol was hydrogenated at 1 atm hydrogen pressure over 30 mg of 10% palladium on carbon for 3 h. The catalyst was removed by filtration through Celite, and the filtrate was
10 concentrated. Trituration with t-butyl methyl ether gave 110 mg (99%) of N-(2R-(t-butoxycarbonyl)methyl-5-(fluoren-2-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)-carboxamide as a white solid: mp 166-167 °C (softening at 161 °C).

15 El. Anal. Calc. for C₃₇H₄₆N₂O₆: C, 72.29; H, 7.54, N, 4.56.
Found: C, 72.32; H, 7.54; N, 4.62.

42B. Formula (Ib') Varying R²

By following the procedure of part A and substituting N-(2R-(t-butoxycarbonyl)methyl-5-(fluoren-2-yl)-4-pentenoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide with:
20 N-(2R-(t-butoxycarbonyl)methyl-5-(7-(N-(benzyloxy-carbonyl)glycyl)aminofluoren-2-yl)-4E-pentenoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide; and
25 N-(2R-(t-butoxycarbonyl)methyl-5-(4-(pyrid-4-yl)phenyl)-4E-pentenoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)-carboxamide,

there are obtained:

30 N-(2R-(t-butoxycarbonyl)methyl-5-(7-(glycyl)aminofluoren-2-yl)pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide:
FAB-MS (M+H)⁺ calculated for C₄₀H₅₁N₄O₇: 699.3758;
observed: 699.3770; and
35 N-(2R-(t-butoxycarbonyl)methyl-5-(4-(pyrid-4-yl)phenyl)-pentanoyl)-L-leucine-N'-(4-(methoxycarbonyl)phenyl)carboxamide: mp 174-176 °C; ¹H NMR (300 MHz,
CDCl₃) δ 8.85 (s, 1H), 8.63 (d, 2H, J = 5 Hz), 7.95 (d, ..

-113-

2H, J = 8 Hz), 7.57 (d, 2H, J = 9 Hz), 7.41-7.47 (m, 4H),
7.07 (d, 2H, J = 8 Hz), 6.19 (d, 1H, J = 7 Hz), 4.53-4.56
(m, 1H), 3.85 (s, 3H), 2.37-2.66 (m, 5H), 1.45-1.83 (m,
7H), 1.42 (s, 9H), 0.91 and 0.95 (2 d, 6H, J = 7 Hz).

5

Example 43

Formula (C-1) Where R⁴ and R⁵ are H

To a stirred suspension of 4.18 g (20.0 mmol) of N-(benzyloxycarbonyl)glycine, 2.73 mL (2.79 g, 30 mmol) of aniline, and 110 mg (1.0 mmol) of 4-dimethylaminopyridine in 55 mL of dichloromethane at 0 °C was added 6.53 g (22 mmol) of EDC methiodide in one portion. The mixture was stirred for 18 h at room temperature, and then partitioned between 200 mL of 3:1 ethyl acetate:hexane and water. The organic layer was washed with 1 N aq. sodium bisulfate, sat. aq. sodium bicarbonate, and finally with brine/pH 7 phosphate buffer, dried over sodium sulfate, and concentrated. Recrystallization from 1:1 ethyl acetate/isooctane gave 3.69 g (65%) of N-(benzyloxycarbonyl)glycine-N'-phenylcarboxamide: mp 143-144 °C.

Example 44

Formula (C-2) Where R⁴ and R⁵ are H

To a stirred solution of 1.42 g (5.00 mmol) of N-(benzyloxycarbonyl)glycine-N'-phenylcarboxamide in 35 mL of dry THF at -5 °C was added by syringe 6.15 mL (16.0 mmol) of 2.6 M n-butyllithium in hexane at a rate to maintain the reaction temperature below 10 °C. After ca. 2/3 of the n-butyllithium had been added, a yellow color began to persist, and the addition was stopped for ca. 10 min and then resumed in a dropwise fashion so as to maintain the reaction temperature at about 0 °C. After the addition was complete, the orange solution was stirred at 0 °C for 45 min, and then cooled to -70 °C. Acetone (1.10 mL, 15 mmol) was added in one portion by syringe. After 10 min, the reaction was partitioned between 1 M pH 7 phosphate buffer and 3:1 ethyl acetate:hexane. The organic layer was washed with brine,

-114-

dried over sodium sulfate, and concentrated. The residue was purified by chromatography on 75 g of silica, eluting with 40% ethyl acetate:hexane. First to elute was pure products fractions (pool #1), followed by fractions containing product and the starting glycylanilide (pool #2). The residue from pool #2 was recrystallized from ethyl acetate:isooctane to give nearly pure starting material as a solid, and mother liquors containing mostly product. The residue from the concentration of the mother liquors was purified by radial chromatography (4 mm plate, 30% ethyl acetate:hexane), and the product fractions were combined with pool #1 to give, after trituration of the gummy residue with hexane/t-butyl methyl ether, 423 mg (25%) of N-(benzyloxycarbonyl)-DL- β -hydroxyvaline-N'- (phenyl)carboxamide as a pale yellow solid:
mp 128-129 °C; ^1H NMR (300 MHz, CDCl₃) δ 8.30 (br s, 1H), 7.39 (d, 2H, J = 8 Hz), 7.18-7.28 (m, 7H), 7.06 (t, 1H, J = 7 Hz), 5.83 (br d, 1H), 5.05 (s, 2H), 4.04 (d, 1H, J = 9 Hz), 3.73 (s, 1H), 1.33 (s, 3H), 1.16 (s, 3H).
El. Anal. Calc. for C₁₉H₂₂N₂O₄: C, 66.65; H, 6.48, N, 8.18.
Found: C, 66.66; H, 6.57; N, 8.14.

Example 45

Formula (C-2) Where R⁴ and R⁵ are H

A solution of 400 mg (1.17 mmol) of N-(benzyloxycarbonyl)-DL- β -hydroxyvaline-N'-phenylcarboxamide in 10 mL of ethyl acetate was hydrogenated over 50 mg of 10% palladium on carbon at 1 atm of hydrogen pressure for 1.5 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated to give 259 mg (>100%) of DL- β -hydroxyvaline-N'- (phenyl)carboxamide, which was used without further purification: mp 97-99 °C.
El. Anal. Calc. for C₁₁H₁₆N₂O₂: C, 63.44; H, 7.74, N, 13.45.
Found: C, 63.52; H, 7.79; N, 13.40.

-115-

Example 46

Formula (Ib) Where R² is Biphenyl, R³ is Hydroxy-t-butyl, and R⁴ and R⁵ are H

To a solution of 203 mg (0.55 mmol) of 2R-(t-butoxy-carbonyl)methyl(5-(biphen-4-yl)pentanoic acid, 104 mg (0.50 mmol) of DL- β -hydroxyvaline-N'-(phenyl)carboxamide, and 90 μ L (0.65 mmol) of triethylamine in 2.5 mL of DMF was added 265 mg (0.60 mmol) of benzotriazol-1-yl-tris-(dimethylamino)-phosphonium hexafluorophosphate. After 24 h, the reaction was partitioned between 3:1 ethyl acetate:hexane and ca. 0.2 N aq. sodium bicarbonate. The organic layer was washed with 1N aq. sodium bisulfate and with brine/pH 7 buffer, dried over sodium sulfate, and concentrated. The residue was purified by radial chromatography (4 mm plate), eluting with 25% to 30% ethyl acetate in hexane. First to elute was 121 mg (43%) of N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-D- β -hydroxyvaline-N'-(phenyl)carboxamide (diastereomer), followed by 140 mg (50%) of N-(2R-(t-butoxycarbonyl)methyl-5-(biphen-4-yl)pentanoyl)-L- β -hydroxyvaline-N'-(phenyl)carboxamide as a gummy semi-solid containing, according to NMR analysis, about 1 mole-equivalent of isooctane (the solvent from which the final sample was concentrated): ^1H NMR (300 MHz, CDCl₃) δ 8.77 (s, 1H), 7.24-7.56 (m, 11 H), 7.03-7.14 (m, 3H), 6.89 (d, 1H, J = 8.5 Hz), 4.46 (d, 1H, J = 8.5 Hz), 4.17 (s, 1H), 2.52-2.70 (m, 4H), 2.36 (br d, 1H, J = 12.5 Hz), 1.50-1.70 (m, 4H), 1.43 (s, 3H), 1.40 (s, 9H), 1.25 (s, 3H).

Example 47

Formula (P-1)

To a solution of 510 mg (1.97 mmol) of N-(4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone in 8 mL of dichloromethane at 0 °C was added 2.2 mL (2.2 mmol) of 1 M titanium tetrachloride in dichloromethane. After 15 min, 0.42 mL (2.4 mmol) of diisopropylethylamine was added to the thick slurry to give a deep red solution. After 1 h at 0 °C, 216 mg (2.4 mmol) of s-trioxane in 2 mL of dichloromethane was added via cannula, followed by an additional 2.2 mL of 1M titanium tetrachloride.

-116-

in dichloromethane. After 4 h at 0 °C, the solution was partitioned between aq. ammonium chloride and dichloromethane. The organic layer washed with 1 N aq. HCl, with brine containing pH 7 phosphate buffer, dried over sodium sulfate, and concentrated. The residue was purified by chromatography on 20 g silica, eluting with 30% to 40% ethyl acetate in hexane. Recrystallization of the purified product from t-butyl methyl ether/isooctane provided 404 mg (71%) of N-(2R-hydroxymethyl-4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone:
mp 71-72 °C; ^1H NMR (300 MHz, CDCl_3) δ 7.22-7.37 (m, 5H), 5.78 (dd, $J = 10, 7, 4$, and 3 Hz), 5.03-5.15 (m, 2H), 4.69 (dd, 1H, $J = 10, 6, 4$, and 3 Hz), 4.17-4.24 (m, 2H), 4.02-4.10 (m, 1H), 3.85-3.91 (m, 2H), 3.29 (dd, 1H, $J = 4$ and 3 Hz), 2.82 (dd, 1H, $J = 14$ and 10 Hz), 2.44 (dt, 1H, $J = 14$ and 7 Hz), 2.31 (dd, 1H, $J = 14$ and 7 Hz), 2.17 (br s, 1H).

Example 48

Formula (P-2)

To a suspension of 4.0 g (25.1 mmol) of O-benzyl-hydroxylamine hydrochloride in 50 mL of THF at 0 °C under argon was added 11.4 mL (22.8 mmol) of 2M trimethylaluminum in toluene. After the addition was complete, the solution was allowed to warm to room temperature. After 15 min, this solution was added via cannula to a solution of 2.40 g (8.30 mmol) of N-(2R-hydroxymethyl-4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone in 100 mL of THF at 0 °C under argon. The reaction was stirred for 6 h at 0°C, and then partitioned between 1N HCl/brine and ethyl acetate/diethyl ether. The organic layer was with 1M pH 7 phosphate buffer and with brine, dried over sodium sulfate, and concentrated. The residue was purified by chromatography on silica, eluting with 35% to 45% ethyl acetate in hexane, to give, after elution of 4S-phenylmethyl-2-oxazolidinone, 2.01 g of the product. Recrystallization from ethyl acetate: isooctane provided 1.90 g (97%) of N-benzyloxy-2R-hydroxymethyl-4-pentenamide as a white powder: mp 58-59 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.40 (s, 1H), 7.38 (m, 5H), 5.71 (m, 1H), 5.03-5.06

-117-

(m, 1H), 4.91 (dd, 1H, J = 16 and 12 Hz), 3.73 (m, 1H), 2.20 (m, 2H).

El. Anal. Calc. for C₁₃H₁₇NO₃: C, 66.36; H, 7.28, N, 5.95.

Found: C, 66.15; H, 7.32; N, 5.99.

5

Example 49

Formula (P-3)

To a solution of 1.92 g (8.17 mmol) of N-benzyloxy-2R-hydroxymethyl-4-pentenamide in 10 mL of anhydrous pyridine at 10 0°C was added 1.24 mL (16.3 mmol) of mesyl chloride. After 3 h, the reaction was poured onto ice, and the mixture was partitioned between ethyl acetate and 1-N-aq. sodium bisulfate. The organic layer was washed with additional sodium bisulfate, and the combined aqueous layers were 15 extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated. The residual oil was dissolved in 30 mL of acetone and 3.38 g of powdered potassium carbonate was added. The mixture was heated at reflux for 3 h and then cooled to room temperature. The 20 precipitate was removed by filtration through Celite, and the filter cake was washed well with ethyl acetate. The filtrate was concentrated, and the residue was purified by chromatography on silica, eluting with 25% ethyl acetate in hexane, to provide 1.64 g (93%) of N-benzyloxy-3R-(2-propen-1-yl)-2-azetidinone as a slightly orange oil: ¹H NMR (300 MHz, CDCl₃) δ 7.37-7.42 (m, 5H), 5.65-5.75 (m, 1H), 5.00-5.06 (m, 1H), 4.93 (s, 2H), 3.32 (ddd, 1H, J = 5, 4, and 2 Hz), 2.95 (m, 2H), 2.40-2.47 (m, 1H), 2.20-2.28 (m, 1H).

25 El. Anal. Calc. for C₁₃H₁₅NO₂: C, 71.86; H, 6.96, N, 6.45.

30 Found: C, 71.59; H, 6.88; N, 6.37.

Example 50

Formula (P-4) Where R² is Biphenyl

A solution of 434 mg (2.00 mmol) of N-benzyloxy-3R-(2-propen-1-yl)-2-azetidinone, 583 mg (2.5 mmol) of 4-bromobiphenyl, 0.34 mL (2.5 mmol) of triethylamine, 35 mg (0.11 mmol) of tri(o-tolyl)phosphine, and 14 mg (0.06 mmol) of

-118-

5 palladium diacetate in 7 mL of DMF was heated at 100 °C for 18 h. The reaction solution was cooled to room temperature and partitioned between ethyl acetate and water. The organic layer was washed with water, dried over sodium sulfate, and concentrated. The residue was chromatographed on silica, eluting with 25% ethyl acetate in hexane, to give slightly impure product, which was recrystallized from ethyl acetate/isooctane to give 315 mg (43%) of N-benzyloxy-3R-(3-(biphen-4-yl)-2-propen-1-yl)-2-azetidinone as small white
10 flakes: mp 109-110 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.31-7.61 (m, 14H), 6.43 (d, 1H, J = 15 Hz), 6.18 (ddd, 1H, J = 15, 9, and 7 Hz), 4.94 (s, 2H), 3.36 (dd, 1H, J = 10 and 5 Hz), 3.00-3.04 (m, 2H), 2.60-2.75 (m, 1H), 2.20-2.50 (m, 1H).
15 El. Anal. Calc. for C₂₅H₂₃NO₂: C, 81.27; H, 6.28, N, 3.79. Found: C, 81.09; H, 6.31; N, 3.71.

Example 51

Formula (P-5) Where R² is Biphenyl

20 To a stirred solution of 62.0 mg (0.168 mmol) of N-benzyloxy-3R-(3-(biphen-4-yl)-2-propen-1-yl)-2-azetidinone in 5 mL of 4:1 THF:ethanol was added 2 mL of 1N aq. lithium hydroxide. The mixture was stirred vigorously for 1 h at room temperature, and the diluted with 10 mL of 0.5 M pH 4 citrate buffer. The mixture was partitioned between 20 mL of t-butyl methyl ether and brine, and the organic layer was dried, after dilution with ca. 5 mL hexane, over sodium sulfate, and concentrated to a residual glass. This residue was immediately dissolved in 5 mL of dichloromethane, cooled to 0 °C, and 0.10 mL of pyridine was added, followed by 1.2 mL of a solution of formic anhydride in dichloromethane, which was prepared by allowing 297 mg (1.00 mmol) of EDC methiodide and 80 μL (2.00 mmol) of formic acid in 5 mL of dichloromethane to react at 0°C for 15 min. After 30 min, the reaction was partitioned between dichloromethane and 0.5 M pH 3 citrate buffer. The organic layer was dried over sodium sulfate and concentrated. Chromatography of the residue on 5 g of silica, eluting with a gradient of 5% to 10% ethanol in

-119-

dichloromethane, gave 58 mg (83%) of N-((N"-formyl-N"-benzyloxyamino)methyl-5-(biphen-4-yl)-4-pentenoic acid as a glass. ¹H NMR spectrum at room temperature in CDCl₃, showed broad peaks of amide rotamers.

5

Example 52

Formula (P-6) Where R² is Biphenyl, R³ is t-Butyl, R⁷ is 4-Pyridyl and p is Zero

To a solution of 99.6 mg (0.24 mmol) of N-((N"-formyl-N"-benzyloxyamino)methyl-5-(biphen-4-yl)pentanoic acid, 125 mg (0.288 mmol) of L-t-leucine-N'-(pyridin-4-yl)carboxamide bis(trifluoroacetate), and 0.125 mL (0.90 mmol) of triethylamine in 4 mL of DMF was added 133 mg (0.30 mmol) of benzotriazol-1-yl-tris-(dimethylamino)phosphonium hexafluorophosphate. After 16 h at room temperature, the reaction was partitioned between ethyl acetate and ca. 0.5 M aq. sodium bicarbonate. The organic layer was washed with 1 M pH 7 phosphate buffer and with brine, dried over sodium sulfate, and concentrated. Purification of the residue by chromatography, eluting with 40% to 75% ethyl acetate in hexane, followed by recrystallization from ethyl acetate/isooctane gave 97.4 mg (67%) of N-((N"-formyl-N"-benzyloxyamino)methyl-5-(biphen-4-yl)-4-pentenoyl)-L-t-leucine-N'-(pyridin-4-yl)carboxamide: mp: 215-216 °C

25

Example 53

53A. Formula (Ib) Where R² is Biphenyl, R³ is t-Butyl, R⁷ is 4-Pyridyl and p is Zero

A solution of 87.1 mg (0.143 mmol) of N-((N"-formyl-N"-benzyloxyamino)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(pyridin-4-yl)carboxamide in 5 mL of 3:2 ethyl acetate/ethanol was hydrogenated over 25 mg of 10% palladium on carbon at 1 atm of hydrogen pressure for 6 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated. Recrystallization of the residue from ethyl acetate gave 59.0 mg (80%) of N-((N"-formyl-N"-hydroxy-amino)methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'

-120-

(pyridin-4-yl)carboxamide as a white powder: mp 190-191 °C;
¹H NMR (300 MHz, DMSO-d₆) δ 10.52 (br s, 1H), 9.97 (br s, 1H,
9.53 (br s, 1H), 8.38 (d, 2H, J = 7 Hz), 7.20 (br s, 1H), 7.14
(br d, 1H, J = 8 Hz), 7.23-7.60 (m, 9H), 7.12 (d, 2H, J = 7
Hz), 4.41 (d, 1H, J = 9 Hz) 3.40-3.62 (m, 2H), 2.90-3.10 (m,
1H), 2.4-2.6 (m, 2H, partially obscured by DMSO-d₅ resonance),
1.28-1.52 (m, 4H), 0.94 (s, 9H).
El. Anal. Calc. for C₃₀H₃₆N₄O₄·0.5 H₂O: C, 68.55; H, 7.10, N,
10.66. Found: C, 68.48; H, 7.04; N, 10.63.

10

53B. Formula (Ib) Varying R², R³, and R'

By following procedures analogous to Examples 50-52 there are obtained the following compounds of formula (P-6):

- 15 N- (2R- (N"-formyl-N"-benzyloxyamino)methyl-5- (4- (2RS-hydroxy-
3,3,3-trifluoropropyl)phenyl)-4-pentenoyl)-L-t-leucine-
N'- (pyridin-4-yl)carboxamide;
- 20 N- (2R- (N"-formyl-N"-benzyloxyamino)methyl-5- (4- (imidazol-4-
yl)phenyl)-4-pentenoyl)-L-threonine-N'- ((4S-
methylsulfinyl)phenyl)carboxamide;
- 25 N- (2R- (N"-formyl-N"-benzyloxyamino)methyl-5- (4- (2RS-hydroxy-
3,3,3-trifluoropropyl)phenyl)-4-pentenoyl)-L-t-leucine-
N'- (pyridin-4-yl)carboxamide;
- 30 N- (2R- (N"-formyl-N"-benzyloxyamino)methyl-5- (4- (imidazol-4-
yl)phenyl)-4-pentenoyl)-L-threonine-N'- ((4S-methyl-
sulfinyl)phenyl)carboxamide;
- 35 N- (2R- (N"-formyl-N"-benzyloxyamino)methyl-5- (4- (pyridin-4-
yl)phenyl)-4-pentenoyl)-L-t-leucine-N'- (4- ((2-
hydroxyethyl)-aminosulfonyl)phenyl)carboxamide;
- N- (2R- (N"-formyl-N"-benzyloxyamino)methyl-5- (4- (pyridin-4-
yl)phenyl)-4-pentenoyl)-L-(β-hydroxy)valine-N'- (4S-
methylsulfinyl)phenyl)carboxamide;
- N- (2R,S- (N"-formyl-N"-benzyloxyamino)methyl- (4- (methyl)-4-
pentenoyl)-L-leucine-N'- (4- (methoxycarbonyl)phenyl)-
carboxamide; and
- N- (2R- (N"-formyl-N"-benzyloxyamino)methyl-5- (biphen-4-
yl)pentanoyl)-L-cyclohexylglycine-N'- ((4- ((2- (dimethyl-
amino)ethyl)aminosulfonyl) phenyl)carboxamide,

-121-

which, when substituted for N-((N"-formyl-N"-benzyloxyamino)-methyl-5-(biphen-4-yl)pentanoyl)-L-t-leucine-N'-(pyridin-4-yl)carboxamide in the procedure of Example 53A, give the following respective compounds:

- 5 N- (2R- (N"-formyl-N"-hydroxyamino)methyl-5- (4- (2RS-hydroxy-
 3,3;3-trifluoropropyl)phenyl)pentanoyl)-L-t-leucine-N'-(pyridin-4-
 yl)carboxamide;
- 10 N- (2R- (N"-formyl-N"-hydroxyamino)methyl-5- (4- (imidazol-4-
 yl)phenyl)-pentanoyl)-L-threonine-N'-(4S-
 methylsulfinyl)phenyl)carboxamide;
- 15 N- (2R- (N"-formyl-N"-hydroxyamino)methyl-5- (4- (pyrid-4-
 yl)phenyl)pentanoyl)-L-t-leucine-N'-(4- ((2-
 hydroxyethyl)aminosulfonyl)- phenyl)carboxamide;
- 20 N- (2R- (N"-formyl-N"-hydroxyamino)methyl-5- (4- (pyrid-4-
 yl)phenyl)pentanoyl)-L- (β -hydroxy)valine-N'-(4S-
 methylsulfinyl)phenyl)carboxamide;
- 25 N- (2R, S- (N"-formyl-N"-hydroxyamino)methyl- (4-
 (methyl)pentanoyl)-L-leucine-N'-(4- (methoxycarbonyl)-
 phenyl)carboxamide: MS (M-H): 434.2; (M-CO, H₂O): 388; and
30 N- (2R- (N"-formyl-N"-hydroxyamino)methyl-5- (biphen-4-
 yl)pentanoyl)-L-cyclohexylglycine-N'-(4- ((2-
 (dimethylamino)ethyl)aminosulfonyl)phenyl)carboxamide.

Examples 54-59

- 25 These examples illustrate the preparation of a representative pharmaceutical formulation containing an active compound of formula (I), e.g., N-(2R-carboxymethyl-5-(biphen-4-yl)pentanoyl)-L- β -hydroxyvaline-N'-(phenyl)carboxamide, or a pharmaceutically acceptable salt thereof. Other compounds of formula (I) can be used as the active compound in preparation of the formulations of these examples.

-122-

Example 54

This example illustrates the preparation of representative pharmaceutical formulations for oral administration.

5	A. <u>Ingredients</u>	% wt./wt.
	Compound of formula (I)	20.0%
	Lactose	79.5%
	Magnesium stearate	0.5%

10 The above ingredients are mixed and dispensed into hard-shell gelatin capsules containing 100 mg each, one capsule would approximate a total daily dosage.

15	B. <u>Ingredients</u>	% wt./wt.
	Compound of formula (I)	20.0%
	Magnesium stearate	0.9%
	Starch	8.6%
	Lactose	79.6%
	PVP (polyvinylpyrrolidone)	0.9%

20 The above ingredients with the exception of the magnesium stearate are combined and granulated using water as a granulating liquid. The formulation is then dried, mixed with the magnesium stearate and formed into tablets with an appropriate tabletting machine.

25	C. <u>Ingredients</u>	
	Compound of formula (I)	0.1 g
	Propylene glycol	20.0 g
	Polyethylene glycol 400	20.0 g
30	Polysorbate 80	1.0 g
	Water	q.s. 100 mL

35 The compound of formula (I) is dissolved in propylene glycol, polyethylene glycol 400 and polysorbate 80. A sufficient quantity of water is then added with stirring to provide 100 mL of the solution which is filtered and bottled.

-123-

D.	<u>Ingredients</u>	<u>% wt./wt.</u>
	Compound of formula (I)	20.0%
	Peanut Oil	78.0%
	Span 60	2.0%

5 The above ingredients are melted, mixed and filled into soft elastic capsules.

Example 55

10 This example illustrates the preparation of a representative pharmaceutical formulation for parenteral administration.

Ingredients

	Compound of formula (I)	0.02 g
	Propylene glycol	20.0 g
15	Polyethylene glycol 400	20.0 g
	Polysorbate 80	1.0 g
	0.9% Saline solution	q.s. 100 mL

20 The compound of formula (I) is dissolved in propylene glycol, polyethylene glycol 400 and polysorbate 80. A sufficient quantity of 0.9% saline solution is then added with stirring to provide 100 mL of the I.V. solution which is filtered through a 0.2 μ membrane filter and packaged under sterile conditions.

25

Example 56

This example illustrates the preparation of a representative pharmaceutical composition in suppository form.

30	<u>Ingredients</u>	<u>% wt./wt.</u>
	Compound of formula (I)	1.0%
	Polyethylene glycol 1000	74.5%
	Polyethylene glycol 4000	24.5%

35 The ingredients are melted together and mixed on a steam bath, and poured into molds containing 2.5 g total weight.

-124-

Example 57

This example illustrates the preparation of a representative pharmaceutical formulation for insufflation.

	<u>Ingredients</u>	<u>% wt./wt.</u>
5	Micronized compound of formula (I)	1.0%
	Micronized lactose	99.0%

The ingredients are milled, mixed, and packaged in an insufflator equipped with a dosing pump.

10

Example 58

This example illustrates the preparation of a representative pharmaceutical formulation in nebulized form.

	<u>Ingredients</u>	<u>% wt./wt.</u>
15	Compound of formula (I)	0.005%
	Water	89.995%
	Ethanol	10.000%

20 The compound of formula (I) is dissolved in ethanol and blended with water. The formulation is then packaged in a nebulizer equipped with a dosing pump.

Example 59

25 This example illustrates the preparation of a representative pharmaceutical formulation in aerosol form.

	<u>Ingredients</u>	<u>% wt./wt.</u>
	Compound of formula (I)	0.10%
	Propellant 11/12	98.90%
	Oleic acid	1.00%
30	The compound of formula (I) is dispersed in oleic acid and the propellants. The resulting mixture is then poured into an aerosol container fitted with a metering valve.	

Example 60

35 *In vitro* matrilysin assay

Matrilysin was purified from cloned mammalian cell culture by Blue-Sepharose and zinc-chelating sepharose column

-125-

followed by fast protein liquid chromatography over a MONO S column. The enzyme was activated by incubation with 1 mmol APMA for 1 hr at 35-37°C.

Compounds of formula (I) were dissolved in DMSO and added 5 to a cuvette containing 0.4 µg matrilysin in 1 ml TC buffer (20mM Tris, 5mM CaCl₂, pH 7.5) (2% DMSO final concentration). The concentrations of the compounds of formula (I) were chosen such that there was at least one data point for every 20% change in activity. Enzyme and compounds were permitted to 10 pre-incubate 3 min at 37°C. To initiate the reaction, N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide ("DACM") (Sigma) and thiopeptide (Ac-Pro-Leu-Gly-S-*"Leu"*-Leu-Gly-OEt, Bachem Bioscience Inc.) were added to 20 µM each. The fluorescence increase was recorded with excitation and emission wavelengths 15 of 395 and 485 nm, respectively. Each data point is the average of duplicate experiments. At least six data points, expressed as change in fluorescence per minute versus compound concentration were analyzed using the IC₅₀ fit in the program, Enzfitter.

Compounds of formula (I) exhibited the ability to inhibit 20 matrilysin when tested in this assay.

Example 61

In vitro assay

This assay determines if the compounds of formula (I) 25 inhibit the release of ³⁵S-labelled glycosaminoglycans (GAG's) from cartilage explants.

Small cartilage explants (3 mm diameter) were prepared from freshly sacrificed bovine knee joints and labeled with 30 ³⁵SO₄. ³⁵S-labelled glycosaminoglycans (GAG's) are released into the culture medium in response to the addition of rhIL-1-alpha, which induces the expression of chondrocyte matrix metalloproteases (MMP's), including stromelysin and collagenase. The percent inhibition of labeled GAG's was 35 corrected for spontaneous release in the absence of rhIL-1-alpha. Results for each group represent the mean ± the S.E.M. for five explants.

-126-

Compounds of formula (I), when tested in this assay, displayed the ability to inhibit the release of ³⁵S-labelled GAG's from cartilage explants.

5

Example 62

In vitro assay

An in vitro fetal rat long bone model was used to study the anti-bone resorptive effect of the compounds of formula (I). Bovine PTH was used to induce bone resorption in vitro. The bone resorptive effects were expressed by the amounts of ⁴⁵Ca released from the ⁴⁵Ca pre-labelled fetal rat long bones into the culture medium. The inhibitory effect of the compounds of formula (I) against bovine PTH induced bone resorption was expressed as mean percent inhibition ± sem.

15 ⁴⁵Ca-prelabelled fetal rat long bones (from forearms) were dissected and cultured in Linbro dishes at 37°C overnight BGJb medium, supplemented with 1mg/ml BSA. There were five pairs of bones in each group. The compounds of formula (I) were dissolved in ethanol first, then diluted to various
20 concentrations and added simultaneously with Bovine PTH (1-34) at 1×10^{-6} M on Day 1. The ethanol concentrations in the compound solutions were less than 0.05% which did not interfere with the assay. The assay was terminated on Day 6 with one media change on Day 3.

25 At the end of each medium change, the ⁴⁵Ca present in the culture medium was counted. The remaining bones were digested with 0.1N HCl and the ⁴⁵Ca presented in the bone digest was also counted. The results are expressed as % of the total ⁴⁵Ca released from each pair of bones. Bovine PTH at 1×10^{-6} M induces bone resorption to the maximum level which is set as 100% and this concentration was used as standard. The level of base line bone resorption in the presence of medium only was set as 0%. All compound-treated groups were compared with bovine PTH (1-34) at 1×10^{-6} M. The concentration at which a compound inhibited bone resorption by 50% was defined as IC₅₀.

35 Compounds of formula (I) exhibited the ability to inhibit bovine PTH-induced bone resorption in this assay.

-127-

Example 63

In vitro stromelysin assay

63A. Stromelysin enzymatic activity was measured by a
5 resonance energy transfer fluorogenic assay using the MCA
peptide substrate: 7-methoxycoumarin-4-yl-acetyl-pro-leu-gly-
leu-3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl-ala-arg-NH₂.
Cleavage of the substrate at the gly-leu bond results in the
loss of resonance energy transfer to the 2,4-dinitrophenyl
10 group and an increase in fluorescence of the MCA (7-
methoxycoumarin-4-yl-acetyl) group.

The assay was performed at 37°C in buffer containing 50
mM Tricine, pH 7.5, 10mM CaCl₂, 200 mM NaCl, 1% DMSO and 1.4 nM
stromelysin. The concentration of MCA substrate was 10 or 20
15 μM in a final volume of 1.6 ml. In the absence of compounds to
be tested for inhibitory activity, or in the presence of non-
slowbinding inhibitors, fluorescence was measured with Perkin-
Elmer LS-5B and LS-50B spectrofluorimeters with $\lambda_{\text{excitation}}$
= 328 nm and $\lambda_{\text{emission}}$ = 393 nm over a 3 to 5 minute time period
20 and data were fitted to a straight line. For slow-binding
inhibitors, inhibition data were collected for 45 minutes to 1
hour. Steady-state rates of fluorescence change were
calculated by fitting the curve to an equation for a single
exponential decay containing a linear phase, and taking the
25 fitted value of the linear phase as the steady-state rate.
Compounds of formula (I) were tested and found to be active as
inhibitors of MMP activity in this assay.

63B. The MCA assay can also be used with other matrix
metalloproteinases, such as matrilysin or gelatinase A, by
30 substituting 0.063 nM matrilysin or 0.030 nM gelatinase A for
stromelysin.

While the present invention has been described with
reference to the specific embodiments thereof, it should be
35 understood by those skilled in the art that various changes
may be made and equivalents may be substituted without
departing from the true spirit and scope of the invention.

-128-

In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention.

5

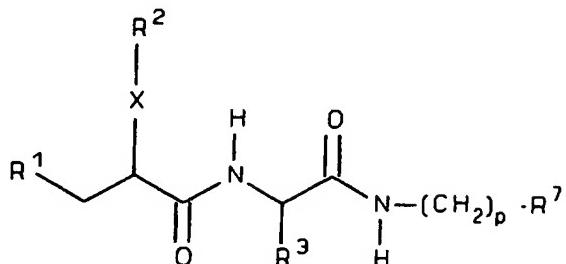
-129-

WHAT IS CLAIMED IS:

1. A compound of the formula

5

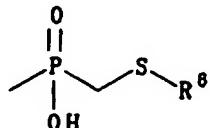
10



wherein:

15 R^1 is mercapto, acetylthio, carboxy, hydroxycarbamoyl, N-hydroxyformylamino, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, benzyloxycarbamoyl or a group of the formula

20



wherein:

25 R^6 is aryl or heteroaryl;
 R^2 is alkyl, cycloalkyl, aryl, heterocycloalkyl, or heteroaryl;
 R^3 is alkyl, cycloalkyl, aralkyl, or heteroaralkyl;
 R^7 is aryl, heteroaryl or heterocycloalkyl;

30 X is a group of the formula $-(CH_2)_m-Y-(CH_2)_n-$, wherein:
 Y is O, S, or a single bond,
 m is an integer from 0 to 4,
 n is an integer from 0 to 4, and
 $m + n$ is an integer from 0 to 4;
 p is an integer from 0 to 4, provided that R^2-X
35 is biphenylalkyl when p is not 0;

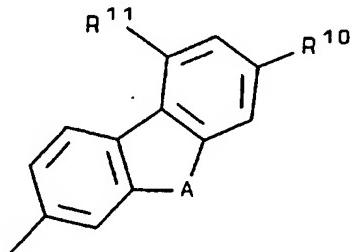
-130-

or a pharmaceutically acceptable salt thereof.

2. The compound or salt of Claim 1 wherein: R¹ is carboxy; R³ is cyclohexyl; R⁷ is optionally substituted phenyl or N-morpholino; X is propane-1,3-diyl; and p is 2 or 3.

5 3. The compound or salt of Claim 2 wherein R⁷ is 4-(aminosulfonyl)phenyl or N-morpholino.

10 4. The compound or salt of Claim 1 wherein: R² is alkyl, optionally substituted phenyl, or a group of the formula:



wherein:

25 A is CH₂, O, NH, S, CH₂-CH₂, or NH-CH₂;
R¹⁰ is H, alkyl, alkoxy, alkylamino or acylamino; and
R¹¹ is H or halo;
R⁷ is 4-pyridyl or optionally substituted phenyl; and
p is 0.

30 5. The compound or salt of Claim 4 wherein: R¹ is carboxy, hydroxycarbamoyl, or N-hydroxyformylamino; R² is phenyl, biphenyl, 4-(pyridyl)phenyl, or 2-methylpropyl; R³ is t-butyl, 4-aminobutyl, dimethylaminobutyl, 4-(N,N'-diethylguanido)butyl, propyl, 2-methylpropyl, 1-hydroxyisopropyl, 1-hydroxyethyl, or cyclohexyl; and X is a single bond, ethylene or propane-1,3-diyl.

-131-

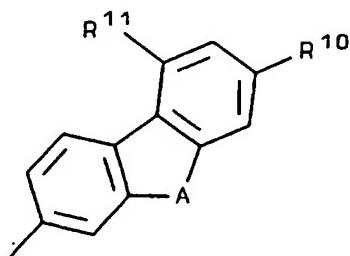
6. The compound or salt of Claim 4 wherein R² is biphenyl.

5 7. The compound or salt of Claim 6 wherein: R¹ is carboxy, N-hydroxyformylamino, or hydroxycarbamoyl; R³ is alkyl; and R⁷ is 4-pyridyl.

10 8. The compound or salt of Claim 7 wherein R³ is t-butyl and X is propane-1,3-diyl.

9. The compound or salt of Claim 8 wherein R¹ is carboxy, N-hydroxyformylamino or hydroxycarbamoyl.

15 10. The compound or salt of Claim 4 wherein: R² is a group of the formula:



25 wherein:

A is CH₂;

R¹⁰ is H or acylamino; and

R¹¹ is H;

R⁷ is optionally substituted phenyl; and

30 X is propane-1,3-diyl.

11. The compound or salt of Claim 10 wherein: R¹ is carboxy, hydroxycarbamoyl, or N-hydroxyformylamino; R³ is alkyl; and R⁷ is alkoxy carbonylphenyl.

35

12. The compound or salt of Claim 11 wherein: R¹ is carboxy; R³ is 2-methylpropyl; and R⁷ is

-132-

4- (methoxycarbonyl)phenyl.

13. The compound or salt of Claim 6 wherein R⁷ is
optionally substituted phenyl.

5

14. The compound or salt of Claim 13 wherein R¹ is
carboxy;
R³ is alkyl or cycloalkyl; and X is propane-1,3-diyl.

10 15. The compound or salt of Claim 14 wherein
R³ is cyclohexyl; and R⁷ is 4-(hydroxyethylaminosulfonyl)phenyl
or 4-(dimethylaminoethylaminosulfonyl)phenyl; or
R³ is 4-(amino)butyl or 4-(isopropylamino)butyl; and R⁷ is
4-(ethoxycarbonyl)phenyl; or
15 R³ is 1-hydroxyisopropyl; and R⁷ is phenyl; or
R³ is tert-butyl.

20 16. The compound or salt of Claim 15 wherein
R⁷ is 4-(N-morpholinopropylaminosulfonyl)phenyl,
4-(methylaminosulfonyl)phenyl, 4-(hydroxyethyl-
aminosulfonyl)phenyl or 4-(methylsulfinyl)phenyl.

25 17. The compound or salt of Claim 4 wherein: R¹ is
carboxy; R² is phenyl; R³ is alkyl or cycloalkyl; and R⁷ is
optionally substituted phenyl.

30 18. The compound or salt of Claim 17 wherein:
R³ is 4-(amino)butyl; R⁷ is 4-(ethoxycarbonyl)phenyl; and X is
propane-1,3-diyl; or R³ is (N,N'-diethylguanido)N-butyl; R⁷ is
4-(ethoxycarbonyl)phenyl; and X is propylene; or R³ is
cyclohexyl; R⁷ is 4-(N",N"-dimethylaminoethylaminosulfonyl)-
phenyl; and X is ethylene.

35 19. The compound or salt of Claim 4 wherein:
R¹ is mercapto, carboxy, hydroxycarbamoyl, or N-hydroxy-
formylamino;
R² is 2-methylpropyl;

-133-

R³ is alkyl, cycloalkyl, or heteroaralkyl;

R⁷ is optionally substituted phenyl; and

X is a single bond.

5 20. The compound or salt of Claim 19 wherein:

R¹ is hydroxycarbamoyl;

R³ is propyl, 2-methylpropyl, cyclohexyl or 3-methylindolyl;
and

10 R⁷ is 4-(methoxy)phenyl, 4-(carboxy)phenyl, 4-(methoxy-
carbonyl)phenyl or 4-(dimethylaminoethylcarbamoyl)phenyl.

21. The compound or salt of Claim 20 wherein

R⁷ is 4-(dimethylaminoethylcarbamoyl)phenyl or

R⁷ is 4-(methoxycarbonyl)phenyl.

15

22. The compound or salt of Claim 19 wherein:

R¹ is N-hydroxyformylamino; R³ is 2-methylpropyl; and R⁷ is
4-(methoxycarbonyl)phenyl; or

R¹ is carboxy; R³ is cyclohexyl or 2-methylpropyl; and R⁷ is 4-
(methoxycarbonyl)phenyl; or

20 R¹ is mercapto; R³ is 2-methylpropyl; and R⁷ is 4- (methoxy-
carbonyl)phenyl.

23. The compound or salt of Claim 4 wherein:

25 R¹ is carboxy;

R² is 4-(2-hydroxyethyl)phenyl, 4-(2-hydroxypropyl)phenyl,
4-(2-hydroxybutyl)phenyl, 4-pyridylphenyl, biphenyl,
4'- (aminoethoxy)biphenyl, 4'- (cyano)biphenyl, or
4'- (hydroxy)biphenyl;

30 R³ is 2-methylpropyl;

R⁷ is 4-(methoxycarbonyl)phenyl; and X is propane-1,3-diyl.

24. The compound or salt of Claim 23 wherein R² is
4-(pyridyl)phenyl.

35

25. A pharmaceutical composition comprising a compound
or salt of Claim 1 and a pharmaceutically acceptable

-134-

excipient.

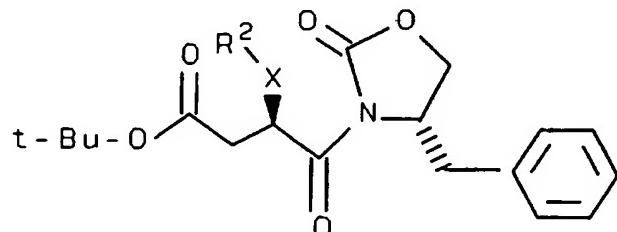
26. A method of inhibiting matrix metalloproteinase activity in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a compound or salt of Claim 1.

27. A method of treating matrix metalloproteinase-mediated disorders selected from the group consisting of arthritis, abnormal wound healing, tissue ulceration, bone resorption disease, diabetes, tumor invasion, tumor metastasis and periodontal disease comprising administering to a mammal in need thereof a therapeutically effective amount of a compound or salt of Claim 1.

15

28. A compound of the formula:

20



25

wherein:

R² is alkyl, aryl or heteroaryl; and

X is a group of the formula - (CH₂)_m-Y- (CH₂)_n,

wherein:

Y is O, S, or a single bond,

30

m is an integer from 0 to 4,

n is an integer from 0 to 4, and

m + n is an integer from 0 to 4;

or R² and X together are lower alkenyl.

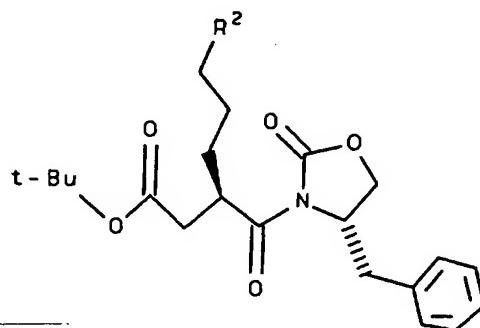
35

-135-

29. A process for making a compound of the formula:

5

10

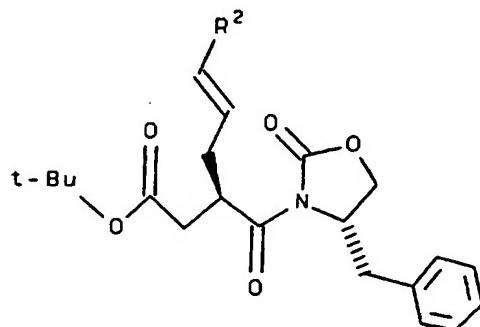


15

wherein R² is aryl or heteroaryl, said process comprising hydrogenating a compound of the formula:

20

25



30

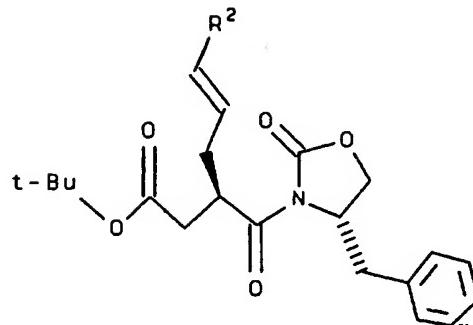
in the presence of a palladium/carbon catalyst.

-136-

30. The process of Claim 29 wherein said compound of the formula:

5

10



15

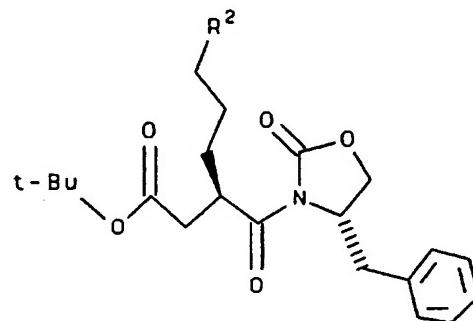
is made by contacting N-(2R-(t-butoxycarbonyl)methyl-4-pentenoyl)-4S-phenylmethyl-2-oxazolidinone with an R²-halide in the presence of a base and a palladium catalyst.

20

31. A process for making a compound of the formula:

25

30



wherein R² is aryl or heteroaryl, said process comprising

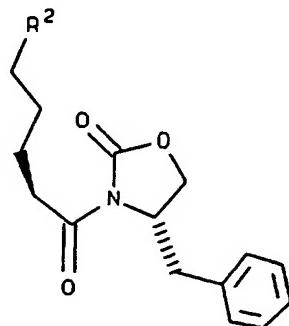
35

-137-

contacting a compound of the formula:

5

10

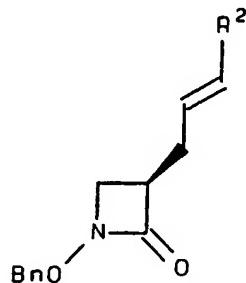


with sodium hexamethyldisilazide-and t-butylbromoacetate.

32. A compound of the formula:

15

20



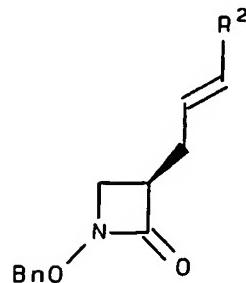
25

wherein R² is aryl or heteroaryl.

33. A process for making a compound of the formula:

30

35



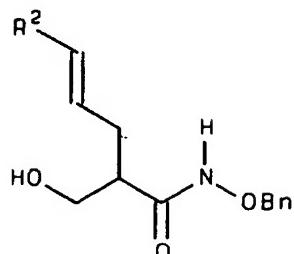
wherein R² is aryl or heteroaryl, said process comprising:

SUBSTITUTE SHEET (RULE 26)

-138-

(a) contacting a compound of the formula:

5



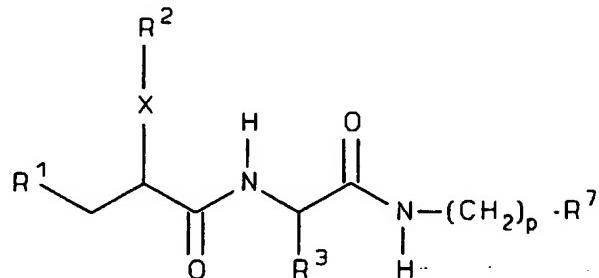
10

wherein R^2 is hydrogen, aryl or heteroaryl,
with an excess of mesyl chloride in pyridine followed by
refluxing under basic conditions, and

15 (b) where R^2 is hydrogen in step (a), reacting the
product of step (a) with an aryl halide or a heteroaryl halide
in the presence of a base and a palladium catalyst.

20 34. A process for the preparation of a compound of
formula (I)

25



30

formula (I)

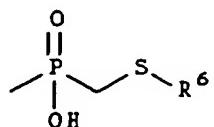
wherein:

35 R^1 is mercapto, acetylthio, carboxy, benzyloxycarbamoyl,
hydroxycarbamoyl, N-hydroxyformylamino,
alkoxycarbonyl, aralkoxycarbonyl,
benzyloxycarbamoyl,

-139-

or a thiomethylphosphinoyl group of the formula

5



wherein: R⁶ is aryl or heteroaryl;

R² is alkyl, aryl, or heteroaryl;

10 R³ is alkyl, cycloalkyl, aralkyl or heteroaralkyl;

X is a group of the formula -(CH₂)_m-Y-(CH₂)_n,

wherein:

Y is O, S, or a single bond,

m is an integer from 0 to 4,

15 n is an integer from 0 to 4, and

m+n is an integer from 0 to 4;

R⁷ is aryl, heteroaryl or heterocycloalkyl; and

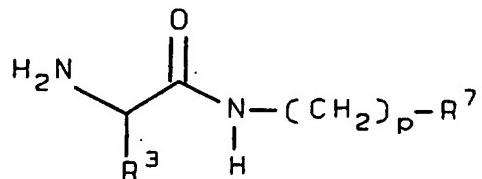
p is an integer from 0 to 4;

said process comprising:

20

(A) contacting a compound of formula (D)

25



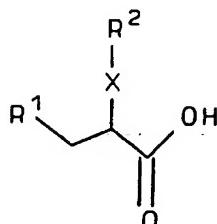
30 (D)

35

-140-

with a compound of formula (F)

5



(F)

10

15

wherein R¹ is alkoxy carbonyl, aralkoxy carbonyl, aryl- or heteroaryl-thiomethylphosphinoyl, or acetylthio; in the presence of a base and an amide coupling reagent to give the corresponding compound of formula (I); or

20

(B) catalytically hydrogenating the corresponding compound where X and R² together are optionally aryl- or heteroaryl-substituted alkenyl; or

25

(C) treating a compound of formula (I), where R¹ is alkoxy carbonyl or aralkoxy carbonyl, under mild acidic conditions to give the corresponding compound of formula (I) where R¹ is carboxy; or

30

(D) contacting a compound of formula (I), where R¹ is carboxy, with O-benzylhydroxylamine to give the corresponding compound of formula (I) where R¹ is benzyloxycarbamoyl; or

35

(E) catalytically hydrogenating a compound of formula (I), where R¹ is benzyloxycarbamoyl, to give the corresponding compound of formula (I) where R¹ is hydroxycarbamoyl; or

(F) contacting a compound of formula (I), where R¹ is carboxy, with hydroxylamine to give the corresponding compound

of formula (I) where R¹ is hydroxycarbamoyl; or

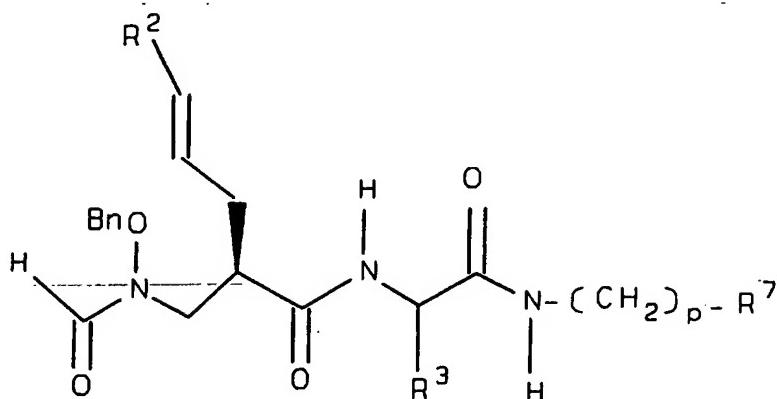
-141-

(G) catalytically hydrogenating a compound of the formula

5

10

15



wherein BnO is benzyloxy, to give the corresponding compound of formula (I) where R¹ is N-hydroxyformylamino; or

20

(H) treating a compound of formula (I), wherein R¹ is acetylthio, with ammonium hydroxide in a protic solvent to give the corresponding compound of formula (I) where R¹ is mercapto.

INTERNATIONAL SEARCH REPORT

Intern. Appl. Application No
PCT/US 95/15530

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C259/06 C07C237/22 C07F09/60 C07C327/30 C07C323/60
C07D263/26 A61K31/16 A61K31/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07C C07F C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 489 579 (CELLTECH LIMITED) 10 June 1992 see claim 1	1
Y	see page 2, line 29 - line 30; claims 1-7	1-27
X	--- WO,A,94 25434 (CELLTECH LIMITED) 10 November 1994 see claim 1	1
Y	see page 2, line 6 - line 14; claims 1-11	1-27
X	--- EP,A,0 438 223 (BEECHAM GROUP P.L.C.) 24 July 1991 see claim 1	1
Y	see page 4, line 40 - line 45; claims 1-12	1-27
X	* intermediate 6 *	28

	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- 'A' document member of the same patent family

2

Date of the actual completion of the international search

14 March 1996

Date of mailing of the international search report

23.04.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Herz, C

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/15530

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 276 436 (F. HOFFMANN-LA ROCHE & CO.) 3 August 1988 see claim 1	1
Y	see page 10, line 19 - line 21; claims 1-34 ---	1-27
X	WO,A,92 09556 (R. E. GALARDY, D. GROBELNY) 11 June 1992 see claim 1	1
Y	see page 4, line 22 - line 33; claims 1-8 ---	1-27
X	WO,A,92 09563 (R. E. GALARDY, D. GROBELNY) 11 June 1992 see claim 1	1
Y	see page 4, line 28 - page 5, line 5; claims 1-8 ---	1-27
X	EP,A,0 489 577 (CELLTECH LIMITED) 10 June 1992 see claim 1	1
Y	see page 2, line 29 - line 31; claims 1-14 ---	1-27
X	WO,A,92 06966 (BEECHAM GROUP PLC) 30 April 1992 see claim 1	1
Y	see page 7, line 24 - line 35; claims 1-16 ---	1-27
X	WO,A,94 07481 (MERCK & CO., INC.) 14 April 1994 see claim 1	1
Y	see page 1, line 15 - page 2, line 5; claims 1-15 ---	1-27
X	WO,A,90 05716 (BRITISH BIO-TECHNOLOGY LIMITED) 31 May 1990 see claim 1	1
Y	see page 1, line 7 - line 29; claims 1-14 ---	1-27
P,X	WO,A,95 12603 (SYNTEX (U.S.A.)) 11 May 1995 see claim 1	1
P,Y	see page 2, line 14 - line 17; claims 1-16 ---	1-27
P,X	WO,A,95 19956 (BRITISH BIOTECH PHARMACEUTICALS LIMITED) 27 July 1995 see claim 1	1
P,Y	see page 5, line 7 - line 16; claims 1-27 ---	1-27
P,X	WO,A,95 19961 (BRITISH BIOTECH PHARMACEUTICALS LIMITED) 27 July 1995 see claim 1	1
P,Y	see page 6, line 4 - line 10; claims 1-25 ---	1-27
	-/-	

2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/15530

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,95 19965 (GLYCOMED INCORPORATED) 27 July 1995 see claim 1	1
P,Y	see page 5, line 31 - page 6, line 11; claims 1-19 ---	1-27
P,X	WO,A,95 12603 (SYNTHEX (U.S.A.) INC.) 11 May 1995 see claim 1	1
Y,P	see page 2, line 14 - line 17; claims 1-16 ---	1-27
Y	WO,A,93 13741 (GLYCOMED, INC.) 22 July 1993 see page 3, line 13 - line 21; claims 1-10 ---	1-27
P,X	WO,A,95 22966 (SANOFI WINTHROP, INC.) 31 August 1995 see claim 1	1
P,Y	see page 1, line 27 - line 30; claims 1-12	1-27
X,P	see page 11; example 1E ---	28
X	EP,A,0 274 453 (LABORATOIRE ROGER BELLON) 13 July 1988 see claim 1	1
Y	see page 26, line 56 - line 60; claims 1-9 ---	1-27
X	WO,A,93 24449 (CELLTECH LIMITED) 9 December 1993 see claim 1	1
Y	see page 20, line 10 - line 17; claims 1-8 * intermediates 2, 9 *	1-27
Y	see page 20, line 10 - line 17; claims 1-8 * intermediates 2, 9 *	28
Y	EP,A,0 498 665 (BRITISH BIO-TECHNOLOGY LIMITED) 12 August 1992 see example 1B see claims 1-14 ---	1-27
X	see example 1B see claims 1-14 ---	28
Y	WO,A,93 09090 (YAMANOUCHI PHARMACEUTICAL CO., LTD.) 13 May 1993 see claim 1 ---	1-27
X	EP,A,0 341 081 (ONO PHARMACEUTICAL CO., LTD.) 8 November 1989 see claim 1	1
Y	see page 16, line 46 - line 50; claims 1-17 ---	1-27
X	WO,A,94 22309 (GLYCOMED INCORPORATED) 13 October 1994 see claim 1	1
Y	see page 4, line 2 - line 9; claims 1-12 ---	1-27
	-/--	

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 95/15530

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	J. MED. CHEM., vol. 38, no. 14, 1995 pages 2570-2581, M. R. GOWRAVARAM ET AL. 'Inhibition of Matrix Metalloproteinases by Hydroxamates Containing Heteroatom-Based Modifications of the P(1)' Group' * compounds 7a-c, 30 *	1-27
X	see tables 1-3 ---	28
Y,P	BIOORG. MED. CHEM. LETT., vol. 5, no. 4, 1995 pages 337-42, J. SINGH ET AL. 'Relationship between structure and bioavailability in a series of hydroxamate based metalloprotease inhibitors' * entire document *	1-27
Y	BIOORG. MED. CHEM. LETT., vol. 4, no. 23, 1994 pages 2741-2746, J. R. PORTER ET AL. 'Potent and selective inhibitors of gelatinase-A. 1. Hydroxamic acid derivatives' * entire document * * Scheme 1, compound 4 *	1-27
X	---	28
Y	BIOORG. MED. CHEM. LETT., vol. 4, no. 23, 1994 pages 2747-2752, J. R. MORPHY ET AL. 'Potent and selective inhibitors of gelatinase-A 2. Carboxylic and phosphonic acid derivatives' * entire document *	1-27
X	---	28
2	J. MED. CHEM., vol. 36, no. 19, 1993 pages 2788-2800, H. HEITSCH ET AL. 'Renin Inhibitors Containing a Pyridyl Amino Diol Derived C-Terminus' * scheme III, compound 9 *	28
X	TETRAHEDRON LETT., vol. 33, no. 18, pages 2447-2450, G. SHAPIRO, C. CHENGZHI 'Asymmetric synthesis of (+)-pilosinine: a formal synthesis of (+)-pilocarpine' * compound 10 *	28
	---	-/-

INTERNATIONAL SEARCH REPORT

Internal	Application No
PCT/US 95/15530	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 402 646 (ABBOTT LABORATORIES) 19 December 1990 see example 235B -----	28

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No

PCT/US 95/15530

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-489579	10-06-92	AT-T-	120182	15-04-95
		AT-T-	120451	15-04-95
		AU-B-	652793	08-09-94
		AU-B-	9017391	25-06-92
		AU-B-	652596	01-09-94
		AU-B-	9023391	25-06-92
		CA-A-	2073510	04-06-92
		CA-A-	2073513	04-06-92
		DE-D-	69108363	27-04-95
		DE-T-	69108363	31-08-95
		DE-D-	69108529	04-05-95
		DE-T-	69108529	30-11-95
		EP-A-	0489577	10-06-92
		ES-T-	2069833	16-05-95
		WO-A-	9209564	11-06-92
		WO-A-	9209565	11-06-92
		GB-A,B	2255339	04-11-92
		GB-A,B	2255340	04-11-92
		HU-A-	61973	29-03-93
		JP-T-	5503719	17-06-93
		JP-T-	5503720	17-06-93
		US-A-	5300501	05-04-94
<hr/>				
WO-A-9425434	10-11-94	AU-B-	6575394	21-11-94
		CA-A-	2139128	10-11-94
		EP-A-	0648205	19-04-95
		JP-T-	8500124	09-01-96
<hr/>				
EP-A-438223	24-07-91	AU-B-	628707	17-09-92
		AU-B-	6931791	18-07-91
		CA-A-	2034016	16-07-91
		JP-A-	4210966	03-08-92
		US-A-	5190937	02-03-93
<hr/>				
EP-A-276436	03-08-88	AU-B-	606901	21-02-91
		AU-B-	8217687	16-06-88
		CA-A-	1326737	01-02-94
		DE-A-	3786250	22-07-93
		DE-T-	3786250	07-10-93
		ES-T-	2056810	16-10-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No

PCT/US 95/15530

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-276436		IE-B- 62334 IL-A- 84766 JP-A- 63156796 US-A- 4885283 US-A- 5006651 ZA-A- 8709230	25-01-95 27-02-94 29-06-88 05-12-89 09-04-91 15-06-88
WO-A-9209556	11-06-92	US-A- 5183900 US-A- 5189178 AU-B- 661289 AU-B- 9089791 AU-B- 662504 AU-B- 9095891 CA-A- 2096221 EP-A- 0558635 EP-A- 0558648 JP-B- 7080825 JP-T- 6511468 WO-A- 9209563 US-A- 5239078 US-A- 5268384	02-02-93 23-02-93 20-07-95 25-06-92 07-09-95 25-06-92 22-05-92 08-09-93 08-09-93 30-08-95 22-12-94 11-06-92 24-08-93 07-12-93
WO-A-9209563	11-06-92	US-A- 5183900 US-A- 5239078 AU-B- 661289 AU-B- 9089791 AU-B- 662504 AU-B- 9095891 CA-A- 2096221 EP-A- 0558635 EP-A- 0558648 JP-B- 7080825 JP-T- 6511468 WO-A- 9209556 US-A- 5189178 US-A- 5268384 CA-A- 2096225	02-02-93 24-08-93 20-07-95 25-06-92 07-09-95 25-06-92 22-05-92 08-09-93 08-09-93 30-08-95 22-12-94 11-06-92 23-02-93 07-12-93 21-02-93
EP-A-489577	10-06-92	AT-T- 120182 AT-T- 120451	15-04-95 15-04-95

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/15530

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-489577		AU-B- 652793 AU-B- 9017391 AU-B- 652596 AU-B- 9023391 CA-A- 2073510 CA-A- 2073513 DE-D- 69108363 DE-T- 69108363 DE-D- 69108529 DE-T- 69108529 EP-A- 0489579 ES-T- 2069833 WO-A- 9209564 WO-A- 9209565 GB-A,B 2255339 GB-A,B 2255340 HU-A- 61973 JP-T- 5503719 JP-T- 5503720 US-A- 5300501	08-09-94 25-06-92 01-09-94 25-06-92 04-06-92 04-06-92 27-04-95 31-08-95 04-05-95 30-11-95 10-06-92 16-05-95 11-06-92 11-06-92 04-11-92 04-11-92 29-03-93 17-06-93 17-06-93 05-04-94

WO-A-9206966	30-04-92	AU-B- 642046 AU-B- 8660491 CA-A- 2092414 EP-A- 0552264 JP-T- 6501944 ZA-A- 9108074	07-10-93 20-05-92 12-04-92 28-07-93 03-03-94 13-04-93

WO-A-9407481	14-04-94	AU-B- 5292193	26-04-94

WO-A-9005716	31-05-90	AU-B- 641629 AU-B- 4746890 CA-A- 2003719 DE-D- 68913988 DE-T- 68913988 EP-A- 0445206 ES-T- 2063334 JP-T- 4503057 NO-B- 177700 US-A- 5304604	30-09-93 12-06-90 23-05-90 21-04-94 28-07-94 11-09-91 01-01-95 04-06-92 31-07-95 19-04-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No

PCT/US 95/15530

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO-A-9512603	11-05-95	AU-B-	8089794	23-05-95
WO-A-9519956	27-07-95	AU-B-	1459795	08-08-95
WO-A-9519961	27-07-95	AU-B-	1460395	08-08-95
WO-A-9519965	27-07-95	AU-B- CA-A- EP-A-	1604995 2158760 0690841	08-08-95 27-07-95 10-01-96
WO-A-9512603	11-05-95	AU-B-	8089794	23-05-95
WO-A-9313741	22-07-93	US-A- AU-B- EP-A- JP-T-	5268384 3433293 0663823 7503007	07-12-93 03-08-93 26-07-95 30-03-95
WO-A-9522966	31-08-95	AU-B-	1881795	11-09-95
EP-A-274453	13-07-88	FR-A- JP-A- US-A-	2609289 63258449 4918105	08-07-88 25-10-88 17-04-90
WO-A-9324449	09-12-93	AU-B- CA-A- EP-A- JP-T-	4341493 2114622 0605682 6509814	30-12-93 09-12-93 13-07-94 02-11-94
EP-A-498665	12-08-92	AU-B- CA-A- WO-A- JP-T- NZ-A- US-A- US-A- ZA-A-	1194492 2100661 9213831 6506445 241558 5412145 5300674 9200908	07-09-92 08-08-92 20-08-92 21-07-94 26-08-94 02-05-95 05-04-94 09-08-93
WO-A-9309090	13-05-93	JP-A- AU-B- CA-A-	5125029 2799792 2122046	21-05-93 07-06-93 13-05-93

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No

PCT/US 95/15530

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9309090		CN-A- 1073166 EP-A- 0613883 US-A- 5442110 US-A- 5473100	16-06-93 07-09-94 15-08-95 05-12-95
EP-A-341081	08-11-89	AT-T- 124683 CA-A- 1330613 DE-D- 68923320 DE-T- 68923320 JP-C- 1849523 JP-A- 2209861 JP-B- 6092358 JP-A- 6179649 US-A- 5017589 US-A- 5202340 US-A- 5317021	15-07-95 05-07-94 10-08-95 14-12-95 07-06-94 21-08-90 16-11-94 28-06-94 21-05-91 13-04-93 31-05-94
WO-A-9422309	13-10-94	AU-B- 6554294 CA-A- 2160139 EP-A- 0692931	24-10-94 13-10-94 24-01-96
EP-A-402646	19-12-90	US-A- 5142056 AU-B- 645493 AU-B- 5571190 CA-A- 2017252 JP-A- 3128335 NZ-A- 233717 US-A- 5362912 US-A- 5354866 US-A- 5151438 US-A- 5256677	25-08-92 20-01-94 29-11-90 23-11-90 31-05-91 25-02-94 08-11-94 11-10-94 29-09-92 26-10-93

THIS PAGE BLANK (USPTO)